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Heteroxylans from maize bran in aqueous solution. Part II: Studies of polyelectrolyte behaviour

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Potentiometric and viscosimetric behaviours of four samples of heteroxylans, extracted from corn bran at different temperatures with varying type and concentration of alkali were studied in dilute solutions. All four samples behave as typical polyelectrolytes with a low charge density parameter ($\lambda = 0.15$). They could be described by the Lifson and Katchalsky model ($pK_0 \approx 3.15$), indicating a rather homogeneous repartition of the charges along the macromolecules. Their intrinsic viscosities varied linearly with the reciprocal of the square root of the ionic strength. Their flexibility parameters, B (≈ 0.024), were similar to those of semi-flexible macromolecules, and their intrinsic viscosities extrapolated to infinite ionic strength were between 144 and 177 ml/g. Their conformational dimensions depended on the type of counter-ion in solution (dimensions minimal with divalent cations and decreasing with size of monovalent alkali metal ions), of the temperature and the pH of the solution. The radius of gyration for unchanged heteroxylans was estimated from intrinsic viscosities extrapolated to infinite ionic strength ($< R^2_{g\infty} >^{1/2} = 21$–$25$ nm). © 1997 Elsevier Science Ltd

INTRODUCTION

Heteroxylans are cell wall polysaccharides which represent about 40% of the maize bran. They mainly consist of a backbone of β-(1-4)-linked D-xylose residues highly substituted by single arabinose residues, glucuronic acid residues, and short side chains of two to three sugar residues (containing arabinose, xylose and galactose). Due to the presence of glucuronic acid (~10%) as side-chain components heteroxylans are weakly charged polyelectrolytes (Watson, 1959; Whistler, 1989; Saulnier *et al.*, 1993). The conditions of alkaline extraction (especially temperature, type and concentration of alkali) greatly influence the yield of solubilized heteroxylans, although they have little influence on their chemical structure (Chanliaud *et al.*, 1995a), macromolecular parameters and conformation in aqueous solution (Chanliaud *et al.*, 1995b). Their radii of gyration are about 40 nm and their hydrodynamic radii are about 20 nm for a weight-average molecular weight of $\approx 2.8 \times 10^5$ g/mol. Light scattering results indicated that heteroxylans had a general random coil behaviour, although a part of the population had a 'compact' structure and another part a more extended conformation.

We now report on the behaviour in dilute solutions of four heteroxylan samples extracted under different conditions. The influence of the solvent (ionic strength and nature of counter-ions), of pH and temperature on viscosity were analysed. From these results and from potentiometric titration study, conformational information was obtained which may help in understanding their functional properties.

EXPERIMENTAL

Heteroxylan samples were extracted as previously described (Chanliaud *et al.*, 1995a). Four sets of extraction conditions were used for 2 h: HX-K100 was extracted by 0.8 N KOH at 100 °C, HX-Ca was extracted by saturated $Ca(OH)_2$ at 100 °C, HX-K60 by 0.5 N KOH at 60°C and HX-N was purified from an industrial liquor (Nejayote) of the lime cooking of maize kernels.

Individual neutral sugars and uronic acids were analysed by gas chromatography and colorimetric

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assays, respectively, as previously described (Chanliaud *et al.*, 1995a).

D-Glucuronic acid was a commercial grade (Fluka, Switzerland). NaOH solutions were prepared just before use to prevent contamination with atmospheric CO_2.

The acidic form of polysaccharides was obtained by precipitation with acidified (0.01 N HCl 96% ethanol (3 vol.; 16 h; 4°C) from heteroxylan in aqueous solution (40 mg/ml). The samples were then extensively washed with ethanol (3 vol.; 96%) until they were chloride-free (silver nitrate test), and dried by solvent exchange. The cation-exchange capacity (CEC) was determined by titration of 100 mg of the heteroxylans in the acidic form with 0.03 N sodium hydroxide. The Na-, K- and Ca-forms were obtained by exact neutralization of the acidic groups, with the chosen hydroxide, NaOH, KOH and $Ca(OH)_2$, respectively.

The pH was measured with a glass electrode and a pHmeter Tacussel (Minisis 8000, France). The dimensionless charge density parameter λ was calculated from the amount of carboxyl groups. Its value at 25°C is given by eqn (1) (Lifson and Katchalsky, 1954)

$$\lambda = \frac{\nu \varepsilon^2}{DhkT} \tag{1}$$

where ε is the electron charge (4.8×10^{-10}), kT the Boltzmann term ($k = 1.38\times10^{-23}$ J/K, $T = 298$ K),υ the number of carboxylic groups per unit length h ($h = n\times h_0$ with n the total number of xylose residues and h_0 the monomeric length = 5 Å; Atkins, 1992) and D the dielectric constant of the solvent (80). Assuming that the main chain of the macromolecule was constituted only of xylose, and that xylose was only present in the main chain of the macromolecule, υ/n was calculated as the ratio of the CEC to the molar fraction of xylose.

From titration curves, the variations of the degree of dissociation (α), were expressed vs the apparent dissociation constant, pK_a. α and pK_a are defined as

$$\alpha = \frac{[RCOO^-]}{[RCOOH]_{total}} = \frac{v}{v_n} + \frac{10^{-pH}(v_i + v)}{v_n[NaOH]} \tag{2}$$

$$pK_a = pH + \log\frac{(1-\alpha)}{\alpha} \tag{3}$$

where v is the volume of NaOH added, v_n the volume of NaOH corresponding to the neutralization and v_i the initial volume. $pK_a(\alpha)$ was determined by extrapolation to $\alpha = 0$ of the linear part of the function $pK_a(\alpha)$ as described by Lifson and Katchalsky (1954). Values were also calculated from the Lifson and Katchalsky (1954)model:

$$pK_a = pK_0 - 0.434\varepsilon\Delta\psi(a)/kT \tag{4}$$

with

$$\Delta\psi(a) = \frac{KT}{\varepsilon}\log\left[\frac{a^2}{R^2}\frac{1-\beta^2}{(\lambda-1)^2-\beta^2}\right] \tag{5}$$

where β is an integration constant. $R = (\pi N C h_0)^{-1/2}$ (C polymer concentration in eq/ml). a was taken as 6 Å which was the average value found for various polyelectrolytes such as pectins (Michel *et al.*, 1984; Racapé *et al.*, 1989), xanthan (Young *et al.*, 1994) or hyaluronates (Cleland *et al.*, 1982).

Viscosity measurements were carried out on heteroxylans at pH 7 and 25°C, in salt solution with an Ubbelohde capillary viscometer (capillary diameter: 0.46 mm). Intrinsic viscosities ([η]) were obtained by extrapolation to zero concentration of the reduced and inherent viscosities obtained for concentrations ranging from 4 to 0.7 mg/ml, using the Huggins and Kraemer equations.

RESULTS

The samples and potentiometric titrations

The chemical characteristics of samples are shown in Table 1. Heteroxylan contents calculated as the sum of xylose, arabinose, galactose and glucuronic acid, represented 88–99% of the samples; the residual glucose was less than 3%. The molar ratios were similar (Xyl:Gal:GlcA:Ara = 1.7:0.3:0.3:1) whatever the samples and their ionic form (native form, Xyl:Gal:GlcA:Ara = 1.7:0.2:0.2:1; Chanliaud *et al.*, 1995a), indicating that probably no degradation occurred during the treatment used to convert heteroxylan to the acidic form.

The cation-exchange capacities of all the samples

Table 1. Chemical and potentiometric characteristics of the acidic heteroxylans (composition is in per cent on a dry weight basis)

	Ara	Xyl	Gal	GlcA	Glc	CEC[a] (mEq/g)	λ[b]	pK_0[c]
HX-K100	27.1	46.9	7.5	6.7	2.0	0.35	0.14	3.05
HX-Ca	29.0	49.9	7.7	7.1	3.0	0.36	0.13	3.05
HX-K60	31.0	52.5	7.8	8.2	2.1	0.40	0.14	3.21
HX-N	27.1	52.7	8.1	6.9	—	0.34	0.12	3.29

[a] Cation-exchange capacity.
[b] Charge density parameter.
[c] Intrinsic apparent dissociation constant.

were similar (Table 1), varying in the range 0.35 (for HX-K100, HX-Ca and HX-N) to 0.40 meq/g (for HX-K60). The quantities of free carboxylic groups (6.1 and 7%, respectively) calculated from these values were close to the amounts of uronic acids measured by the colorimetric assay. The charge density parameters were very similar ($\lambda \sim 0.15$) indicating a behaviour of a weak polyelectrolyte compared to pectins ($\lambda = 0.45$–1.56; Axelos and Thibault, 1991) or alginates ($\lambda = 1.38$; Rinaudo, 1974).

Plots of pK_a vs α are presented in Fig. 1 for glucuronic acid and one heteroxylan (HX-N), all other samples gave the same result (data not shown). As expected, the pK_a values of glucuronic acid showed no significant dependence on α and the mean value of pK was $= 3.02 \pm 0.04$. This value was slightly lower than values reported previously (3.28, Kohn and Kovac, 1978). For heteroxylan samples, the pK_a increased with α, showing that dissociation of each carboxylic group depends on the ionic state of its neighbours. This polyelectrolyte behaviour was tentatively explained by the Lifson and Katchalsky model which was used to fit the pK_a variation vs α, and to extrapolate pK_0 at $\alpha = 0$.

The model was a good fit of pK_a variation when α was lower than 0.7. The pK_0 extrapolated were found between 3.05 and 3.3 (Table 1). These results showed that the heteroxylans followed the Lifson and Katchalsky model although glucuronic acid residues are present as side chains components.

Influence of salt on viscosity

The effect of the addition of inorganic salt on the viscosity of dilute aqueous solutions of heteroxylan in the Na$^+$-form, was studied in order to obtain information about the extension of the molecules. $[\eta]$ varied linearly with the reciprocal square root of the ionic strength ($I^{-1/2}$) for the four samples (Fig. 2) and followed equation (6)

$$[\eta] = [\eta]_\infty + SI^{-1/2} \qquad (6)$$

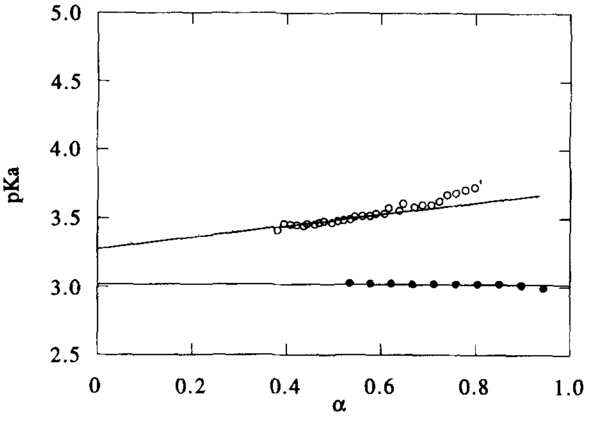

Fig. 1. Dissociation constant of D-glucuronic acid (●) and HX-N (○) vs the degree of dissociation α. The straight line is the representation of the Lifson and Katchalsky model.

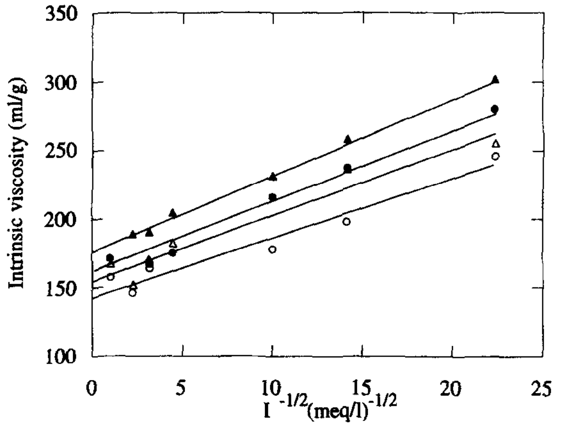

Fig. 2. $[\eta]$ of Na-heteroxylans as a function of the reciprocal of the square root of the ionic strength. ▲: HX-K100; ●: HX-Ca; △: HX-K60; ○: HX-N.

where $[\eta]_\infty$ is the intrinsic viscosity at infinite ionic strength and S the slope which is related to the flexibility of the molecule. The extrapolation to infinite ionic strength might be used for characterizing the properties of polyelectrolytes in an effectively unchanged state. An empirical stiffness parameter B, independent of the molecular weight may be obtained from S using relationship (7) (Smidsrod and Haug, 1971)

$$B = S/([\eta]_{0.1})^{1.3} \qquad (7)$$

where $[\eta]_{0.1}$ (in 100 cm^3/g) is the intrinsic viscosity in 0.1 M NaCl.

The values of B, S and $[\eta]_\infty$ are reported in Table 2. $[\eta]_\infty$ were found between 144 and 177 ml/g. Their values varied in the same way as $<M>_w$. The B values (~ 0.024) were similar for all samples and close to those found for citrus pectins (≈ 0.02) (Axelos and Thibault, 1991) or alginates (≈ 0.04) (Smidsrod and Haug, 1971) which are considered as semi-flexible random coil compared to flexible molecule as amylose ($B = 0.22$; Smidsrod and Haug, 1971) or to rigid macromolecule such as xanthan ($B = 0.005$; Rinaudo and Milas, 1978).

The intrinsic viscosity under different ionic (Na$^+$, K$^+$, Li$^+$ and Ca^{2+}) forms was studied for HX-K100 as a function of the ionic strength of their respective chloride salts (NaCl, KCl, LiCl and CaCl$_2$) (Fig. 3). The intrinsic viscosity decreased only slightly in the presence of CaCl$_2$ indicating a stiffness of the macromolecules through bridges formed by the bivalent ion between the carboxylic groups and/or a very high affinity of the carboxylic groupments for the bivalent ions leading to a decrease of the net charge of the macromolecule. With the other salts, more classical behaviour occurred with a significant decrease of $[\eta]$ with ionic strength. In the range of studied ionic strengths, all of the salts gave a linear dependence for $I^{-1/2}$, and the curves gave the same $[\eta]_\infty$. These results confirmed that the viscosity at infinite ionic strength

Table 2. Physical chemical characteristics of the samples determined by viscosity measurements

	$[\eta]_\infty$ (ml/g)	S	B	$< R_{g\infty}{}^2 >^{1/2}$ (nm)[a]	$R_{g\theta}{}^2 >^{1/2}$ (nm)[b]
HX-K100	176.8	5.68	0.025	25	20
HX-Ca	158.4	4.75	0.024	23	26
HX-K60	160.4	5.13	0.026	23	24
HX-N	144.3	4.22	0.022	21	22

[a] Calculated using equation (10).
[b] Value determined by light scattering (Chanliaud *et al.*, 1996).

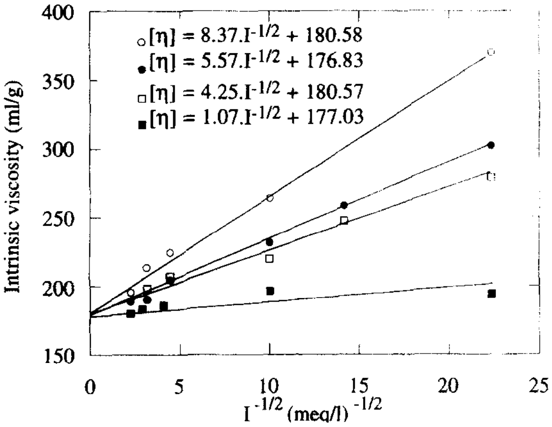

Fig. 3. Relationship of the intrinsic viscosity of HX-K100 and the reciprocal of the square root of the ionic strength for different alkaline metal ions. ○: Potassium; ●: sodium; □: lithium; ■: calcium.

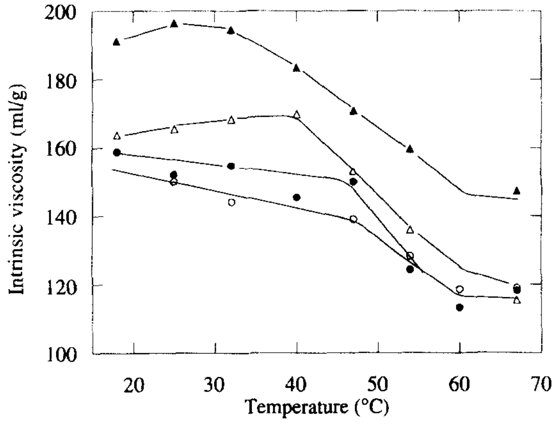

Fig. 4. Intrinsic viscosity of HX-K100 as a function of temperature in different ionic media in NaCl (▲: 10 mM; △: 200 mM) and $CaCl_2$ (●: 10 mM; ○: 200 mM).

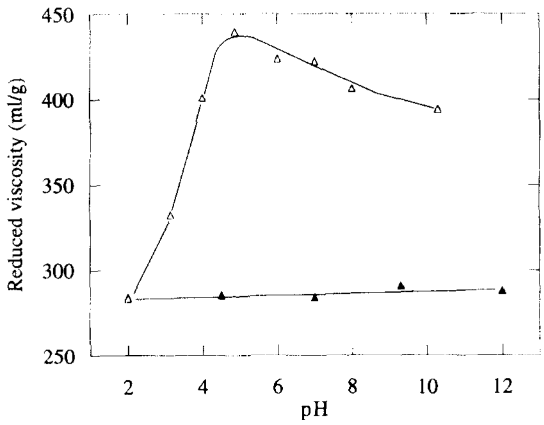

Fig. 5. Reduced viscosity of HX-K100 vs pH of the solution, in pure water (△) and 200 mM (▲).

was representative of the unchanged heteroxylan molecule. The B values calculated from the four slopes (0.025, 0.033, 0.11 and 0.005 with NaCl, KCl, LiCl and $CaCl_2$, respectively) were different, indicating that the macromolecules behave differently according to the type of counter-ions in solution.

Effect of temperature and pH on viscosity

Figure 4 gives the dependence of intrinsic viscosity as a function of temperature under different ionic conditions. In all cases, a decrease of $[\eta]$ was observed with a transition around 50–60°C. The decrease was more noticeable in the presence of NaCl than in the presence of $CaCl_2$ and when the ionic strength was low. This sharp decrease is rather common to the hydrosoluble polysaccharides and proteins. It could indicated a modification in the conformation but more often it is due to a decrease of the expansion of the macromolecule (Axelos and Branger, 1993).

The variation of reduced viscosity versus the pH of the solution is represented in Fig. 5, in the presence or absence of an external salt. Its behaviour was classical for a polyelectrolyte. The salt content and the pH had a marked effect on the viscosity. The viscosity of heteroxylans in salt-free solution was relatively low and increased sharply to a maximum around 5. The viscosity decreased as the pH was further increased and the sodium salt was formed. The ionic strength screened the modification of viscosity due to modification of charge density and expansion.

DISCUSSION

The potentiometric and viscometric results gave information about solution properties and conformation of maize bran heteroxylans. The results were similar for the four samples indicating that the extraction conditions had no effect on the polyelectrolyte behaviour. However, intrinsic viscosities at infinite ionic strength varied significantly (from 144 to 177 ml/g), this variation followed the molecular weight of the samples (3.1, 2.8, 2.7 and 2.4×10^5 g/mol for HX-K100, HX-Ca, KX-K60 and HX-N, respectively) (Chanliaud *et al.*, 1995b).

A value of the radius of gyration can be calculated for the unchanged polysaccharide, e.g. polymer totally screened by infinite ionic strengths, using the Fox–Flory relationship (8)

$$[\eta]_\infty = \Phi \times 6^{3/2} \times < R^2_{g\infty} >^{3/2} / < M > \qquad (8)$$

where Φ is the Fox–Flory constant ($=2.5\times10^{23}$) (Flory, 1953) $<M>$ the weight-average molecular weight and $< R^2_{g\infty} >^{1/2}$, the radius of gyration at infinite ionic strength. The values of $< R^2_{g\infty} >^{1/2}$ calculated were ~23 nm for the four heteroxylans (Table 2). Although the Flory–Fox relationship should be applied only on monodisperse population, these values are in good agreement with the radius of gyration $< R^2_{g\theta} >^{1/2}$ calculated previously for the unperturbed state (Table 2, Chanliaud *et al.*, 1995b), and confirm that maize bran heteroxylans have a general behaviour of random coil. The stiffness parameters B (≈ 0.024) indicated a semi-flexible conformation so that maize bran heteroxylans behaviour might be described as a semi-flexible random coil in solution, confirming the conformational information obtained from light scattering (Chanliaud *et al.*, 1995b).

The viscometric behaviour depends on the type and concentration of cation and temperature. The concentration of external cations in solution modified the intrinsic viscosity of heteroxylans and this modification was more or less marked depending on the nature of the cation. Smidsrod (1970) observed the same phenomenon with alginates. But, in contrast to heteroxylans, the intrinsic viscosity of alginates decreased as the hydration level of alkali metal ions increased from Li^+ and K^+ because the hydrated ions were more tightly bound (Launay *et al.*, 1986). For heteroxylans, the screening of electrostatic repulsions seemed to decrease with the size of the alkali metal ions and therefore the charge density. This phenomenon could be partly involved in the difference of extraction efficiency observed for sodium and potassium hydroxide (Chanliaud *et al.*, 1995a).

The dimensions of the macromolecules in solution were modified by temperature whatever the solvent conditions. This fact probably facilited the extraction of the molecules from bran after their solubilization by alkali. In contrast, the effect of pH on conformation could not occur in the extracting medium because the ionic strength was too high and screened all the charges.

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