AN EXAMINATION OF SOME METHODS FOR FRACTIONATION OF PLANT HEMICELLULOSES

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ABSTRACT

A study has been made of the effectiveness and reproducibility of the fractionation of hemicelluloses by fractional precipitation with ethanol, and by ion exchange on cellulose, dextran, and polystyrene derivatives. Fractionation on a composition basis has been demonstrated by ethanol precipitation. Fractionation on cellulose or on DEAE- or TEAE-cellulose resulted in some sorption of the xylans, which could not be reversed by elution with sodium chloride, and further elution with alkali resulted in slow desorption from the column. This sorption was much reduced with DEAE- and QAE-Sephadex and useful fractionation was achieved, especially of neutral from acidic polysaccharides. A study was also made of the effect of a range of metal ions on the solubility of hemicellulose. An increase in the concentration of metal ions normally caused an initial decrease in solubility, followed by an increase, and the implications of this behaviour are discussed for fractionation procedures involving precipitation by metal complexing.

INTRODUCTION

Hemicelluloses from plants are usually obtained by alkaline extraction of holocelluloses prepared after removal of water-soluble polysaccharides and pectic substances. They can be classified into A and B groups by neutralisation and precipitation, using the method of O'Dwyer[1]. Gaillard[2] has extended this method of classification by using precipitation with iodine to differentiate linear and branched polymers in the B group of hemicelluloses. From this and other work[3], there is tentative evidence suggesting the separate existence in the *Gramineae* of an arabinoxylan and an arabino-4-*O*-methylglucuronoxylan. This latter polysaccharide may be used to distinguish the monocotyledon from the dicotyledon, since 4-*O*-methylglucuronoxylans having very low contents of arabinose are structurally characteristic of the *Leguminosae*.

Recently, we studied the A/B classification of hemicelluloses[4] and have subsequently needed to examine a wide range of techniques for their further fractionation. The results from this range of techniques, particularly with respect to hemicelluloses from Townsville lucerne (*Stylosanthes humilis*), are summarised in this paper.

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EXPERIMENTAL AND RESULTS

Materials. — Hemicelluloses from the tropical grasses, spear grass (*Heteropogon contortus*), guinea grass (*Panicum maximum*), and sugar-cane bagasse, and from the legume Townsville lucerne (*Stylosanthes humilis*) were extracted from a chlorite holocellulose[5] with 10% aqueous sodium hydroxide under nitrogen (water-soluble and pectic components were removed by extraction before the delignification[6]). The hemicelluloses were recovered by neutralization of the alkaline extracts with ion-exchange resin[4], and the B group was precipitated in three volumes of ethanol. The final product was dried by lyophilisation.

No hemicellulose A was found in Townsville lucerne, and the B group was separated into linear and branched polymers by using iodine as outlined by Gaillard[2]. The linear polymer had equivalent weight 1463, $[\alpha]_D^{33}$ −46.2°, and a relative composition ("anhydro sugar") of rhamnose, 2%; arabinose, 3%; xylose, 88%; galactose, 3%; and glucose, 4.5%. The branched polymer (amounting to 3.4% of the hemicellulose) showed an equivalent weight of 861 and $[\alpha]_D^{32}$ +18.4°, and these figures suggest considerable contamination by pectic substances.

A standard procedure used in the preparation of all hemicellulose solutions involved heating at neutral pH for 10 min on a boiling-water bath and then standing at room temperature for 3 h before use[7,8].

Fractional precipitation With ethanol. The branched and linear hemicelluloses from Townsville lucerne were fractionally precipitated with ethanol, as outlined by Whistler and Sannella[9]. Precipitated material was dissolved in M sodium hydroxide and determined by optical rotation of the solution.

The branched polymer failed to precipitate up to a concentration of 87% of ethanol, and was finally fractionated in 10mM calcium chloride. In this instance, the observed optical rotation of precipitated fractions varied from +0.1° to −0.03°, and the fractions having gross positive and negative rotations, respectively, were combined for analysis.

Relative compositions of "anhydro sugar" are shown in Table I. They were

TABLE I

RELATIVE COMPOSITION ("ANHYDRO SUGAR") OF FRACTIONS OBTAINED BY ETHANOL PRECIPITATION OF HEMICELLULOSES FROM TOWNSVILLE LUCERNE (SEE FIG. 1)

Hemicellulose type	*Fraction from precipitation*	*"Anhydro sugar"*				
		Rha	*Ara*	*Xyl*	*Gal*	*Glc*
Linear	I	8	12	51	13	16
	II	1	2	92	2	2
	III	4	5	66	9	17
Branched	(+)	15	21	25	40	—
	(−)	10	25	28	37	—

measured by gas–liquid chromatography (g.l.c.) of the alditol acetates[10]. The precipitation curve for linear xylan is shown in Fig. 1.

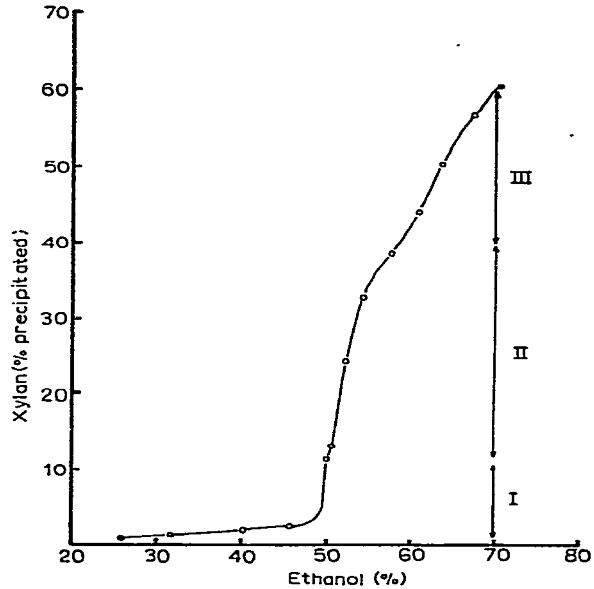


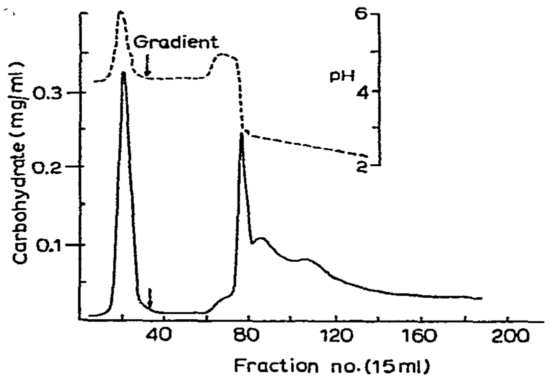
Fig. 1. Fractional precipitation with ethanol of linear xylan from Townsville lucerne.

Ion-exchange chromatography. Diethylaminoethylcellulose (DEAE-C) (Whatman Column Chromedia Grade DE 11) was used for chromatographic fractionation of hemicellulose B from guinea grass.

The powder (80 g) was stirred for 1 h in 0.5M sodium hydroxide (5 l), filtered, and washed with water. The residue was back-washed with deionised water overnight (to remove fines) before two cycles with 0.5M sodium hydroxide and one of 0.5M hydrochloric acid (500–800 ml). In a final cycle, M acetic acid (1.5 l) converted the exchanger into the acetate form.

A slurry was stirred into a glass column (60 × 2.5 cm), and the bed was washed exhaustively with water (7 l) to equilibrate and stabilise it. Hemicellulose B (450 mg in 100 ml) was applied to the column and eluted with water, and the effluent was collected in 15-ml fractions at 1 ml/min. After collection of 30 fractions, a linear gradient to 4M acetic acid was applied during 3 l. Fractions were assayed by the phenol–sulphuric acid procedure[11], and changes in effluent pH were recorded as shown in Fig. 2. Recoveries of 20, 15, and 18% were obtained for fractions *A*, *B*, and *C*, respectively. Similar results were obtained by using TEAE-cellulose (Touzart and Matignon, Paris), as shown in Fig. 3.

Acid–base characteristics of DEAE- and TEAE-cellulose, and Deacidite FF(IP) (2–3% cross-linked, 100–120 mesh) were measured by equilibration to chloride counter-ion, subsequent exhaustive washing, and vacuum drying. Successively larger amounts of 0.1M sodium hydroxide were pipetted into a number of samples (1 g), and



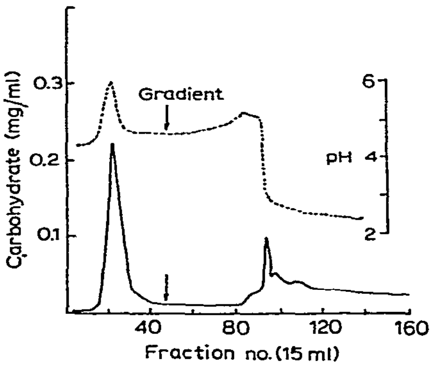


Fig. 2. Fractionation of hemicelluloses from guinea grass on DEAE-cellulose ($^{-}$OAc) with an acetic acid gradient.

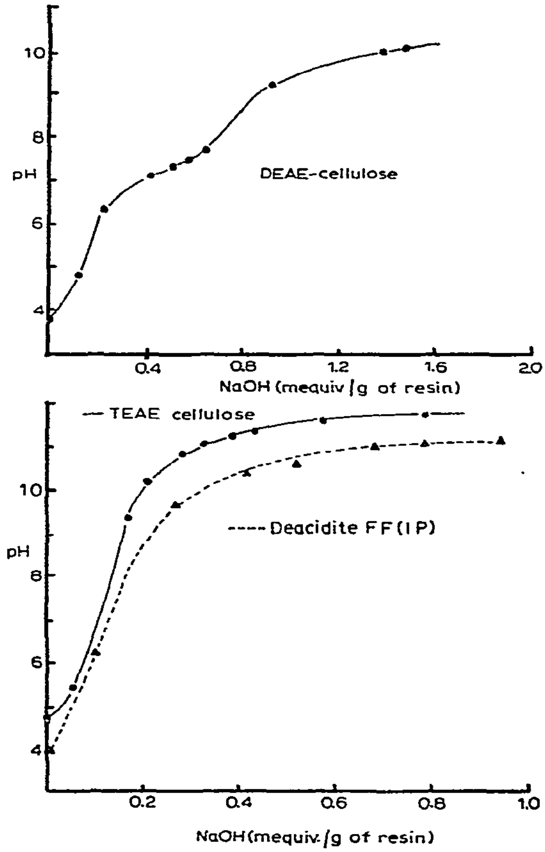
Fig. 3. Fractionation of hemicelluloses from guinea grass on TEAE-cellulose ($^{-}$OAc) with an acetic acid gradient.

deionised water was added to establish a fixed ratio of solution volume/resin weight (30 ml/g). The flasks were stoppered and agitated with occasional shaking over a period of 2 days. The pH of the solution in each sample was determined, and titration curves were obtained by plotting solution pH against added titrant, as shown in Fig. 4.

The sorption effects of linear xylan on cellulose were studied by using Whatman Column Chromedia Grade CF-11 cellulose which was first slurried with water and allowed to settle for 2 days. A portion (8.5 ml) of the sediment was separated and centrifuged, and the residue was again slurried in water (10 ml) and equilibrated at 27.5° before the addition of 1 ml of a 0.2% solution of linear xylan. The vessel was shaken occasionally, and supernatant samples were taken at intervals for colorimetric assay and relation to the original concentration, as shown in Fig. 5. After 5 h, the slurry was transferred to a glass column (10 × 1 cm) and eluted with water (150 ml) to give a recovery of 35% of the original loading.

Assessment of different types of ion-exchange resins was made by using a series of small columns (1-cm diameter) into which measured volumes of the resins (*ca.* 8 ml) were sedimented and equilibrated by elution with 25 mM ammonium acetate. A solution (1 ml, 0.3–0.5%) of linear xylan was applied to each column, and unretained material was collected in 150 ml of effluent. Changes to 3M sodium chloride and 0.5M sodium hydroxide were made, and similar quantities collected. Flow rates were 1–2 ml/min. Colorimetric analysis by the phenol–sulphuric acid procedure enabled determination of relative percentages from a calibration curve prepared from the original, linear xylan.

High recoveries noted in the elution with alkali could not be significantly diminished by prior reduction with sodium borohydride of either the supports or the linear xylan. The results are summarised in Table II.



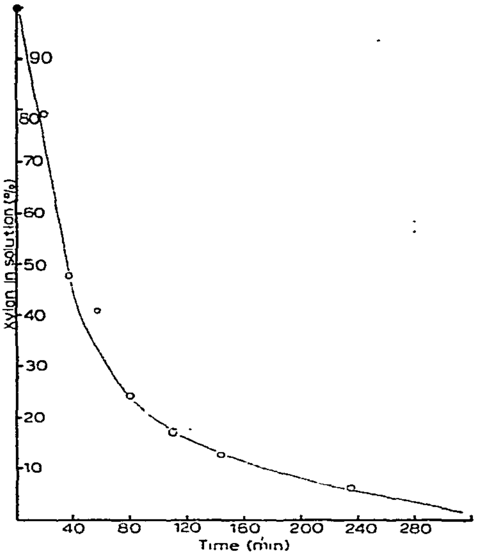


Fig. 4. Acid–base characteristics of DEAE- and TEAE-cellulose, and Deacidite FF(IP); chloride forms titrated with sodium hydroxide.

Fig. 5. Sorption on cellulose of linear xylan from Townsville lucerne in aqueous solution.

TABLE II

RECOVERY (%) OF XYLAN FROM VARIOUS SUPPORTS

Eluent	*Support*						
	Cellulose	*DEAE-C*	*TEAE-C*	*Sephadex G-200*	*DEAE-S*	*QAE-S*	*Deacidite FF(IP)*
25mM Ammonium acetate	79[a] 83[a] 88[a] 40[b] 47[b]	0	0	104	3	12	8
3M Sodium chloride	0	73.5	43 37	—	69	79	—
0.5M Sodium hydroxide[c]	17–105	43–139	92–184	—	1	10–15	2

[a] 1 ml of 0.5% xylan solution added to column. [b] 1 ml of 0.3% xylan solution added to column. [c] The variation in these results was apparently not systematic and is discussed in the text.

Because QAE-Sephadex (QAE-S) seemed most promising, detailed analysis was made of xylan recovery as a function of concentration of the eluting electrolyte. A solution (1 ml, 0.9%) of linear xylan in 25mM ammonium acetate was added to each of six columns (5 × 1 cm) of exchanger equilibrated to the same buffer. After collection of the unretained portion in 150 ml of effluent, changes to a particular concentration of potassium chloride were made, and 150 ml of eluate were collected, as described above. Contractions in bed volumes were recorded, and the results are shown in Table III.

TABLE III

RECOVERY (%) OF LINEAR XYLAN FROM QAE-SEPHADEX A-50 ($^{-}$OAc) FOR DIFFERENT CONCENTRATIONS OF ELUTING ELECTROLYTE

Column No.	*1*	*2*	*3*	*4*	*5*	*6*
Electrolyte concentration (M)	0.05	0.1	0.2	0.4	0.8	1.5
Bed contraction (%)	5.9	10.4	26.2	29.1	42.2	58.2
Unretained xylan	21	22	18	22	20	17
Displaced xylan	17	33	44	44	48	54

A number of studies of fractionation of hemicelluloses on QAE-Sephadex A-50 were made on a larger scale by using gradient elution. The exchanger was prepared[12] in the acetate form and equilibrated and stabilised in a column (final dimensions of 50–60 × 2.1 cm) by pumping 25mM ammonium acetate at 1 ml/min. Xylan was dissolved in starting buffer and loaded and analysed as outlined previously for DEAE-cellulose.

An initial study involved gradient analysis to 20% acetic acid before step-wise changes to 0.5 and 2M sodium hydroxide. The chromatogram is shown in Fig. 6, and a compositional analysis of collected fractions in Table IV.

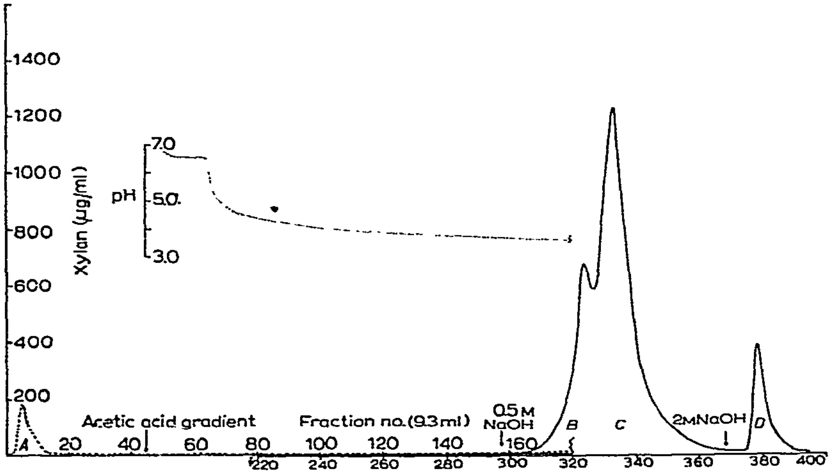


Fig. 6. Fractionation of linear xylan from Townsville lucerne on QAE-Sephadex ($^{-}$OAc) by using an acidic gradient, followed by sodium hydroxide.

TABLE IV

RELATIVE COMPOSITION (NEUTRAL SUGAR) OF FRACTIONS OF LINEAR XYLAN RECOVERED FROM QAE-SEPHADEX ($^-$OAc) (SEE FIG. 7)

Fraction	*"Anhydro sugar"*					
	Rha	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*
A	6	1	28	3	11	52
B	1	1	97	—	1	1
C	1	1	96	1	1	1
D	5	10	70	—	11	4

Because a gradient of acetic acid failed to elute the major portion of the hemicelluloses, a trial of a gradient of ammonium hydroxide (to sp.gr. 0.978) was investigated under similar conditions. Again, no material was eluted by gradient development, but a single sharp change in pH, coincident with those noted for acidic gradients, was recorded. A subsequent change to 0.5M sodium hydroxide gave a chromatogram similar to that in Fig. 6.

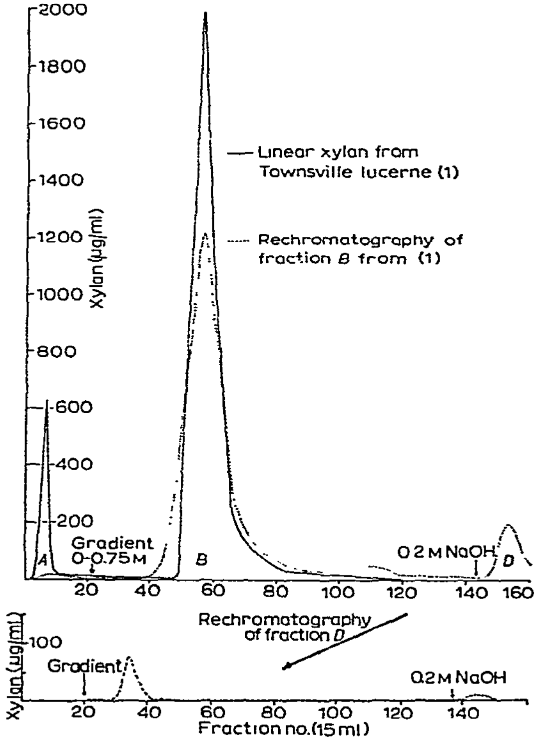


Fig. 7. Fractionation of linear xylan from Townsville lucerne on QAE-Sephadex with a potassium chloride gradient, followed by sodium hydroxide (curve i) and re-chromatography of the major fraction.

Gradients of increasing ionic strength were next investigated. Initial trials involved sharp gradients to 3M potassium chloride (total 3 l), but these were eventually terminated at 0.75M (total 3 l). Fig. 7 shows the effect of re-chromatography on collected fractions *B* (gradient eluted) and *D* (eluted with 0.2M sodium hydroxide subsequent to the gradient).

Figs. 8*a*, *b*, and *c* show chromatograms for hemicellulose preparations from spear grass, guinea grass, and sugar-cane bagasse, respectively, eluted under comparable conditions. The relative compositions "anhydro sugar" for fractions *A* and *B* are given in Table V.

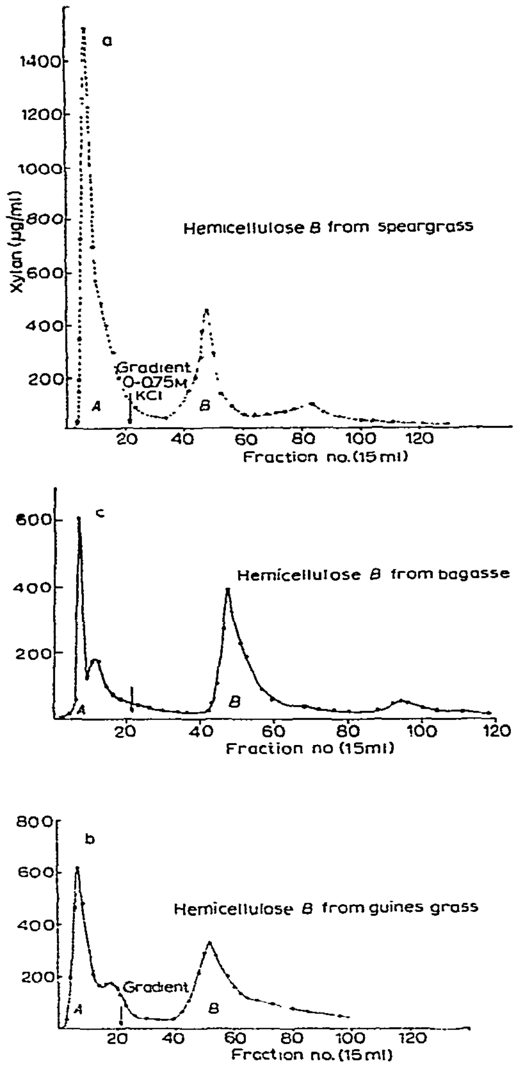


Fig. 8. Comparative chromatograms on QAE-Sephadex ($^{-}$OAc) with a potassium chloride gradient, for various plant hemicelluloses.

TABLE V

COMPOSITION OF FRACTIONS OBTAINED BY CHROMATOGRAPHY OF HEMICELLULOSES ON QAE-SEPHADEX ($^-$OAc) (SEE FIGS. 8*a*, *b*, *c*)

Monosaccharide	*Xylan fraction*							
	Townsville lucerne		*Spear grass*		*Guinea grass*		*Bagasse*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
Rhamnose	2	1	—	—	—	—	—	—
Arabinose	1	1	11	12	12	16	9	12
Xylose	69	95	76	84	62	66	64	76
Mannose	8	—	—	—	—	—	1	—
Galactose	4	1	1	1	1	4	1	1
Glucose	16	1	9	3	26	14	25	9

Free-boundary electrophoresis in borate buffer[13] revealed that fraction *B* of the linear xylan from Townsville lucerne, which was chromatographically pure, contained two components as shown in Fig. 9.

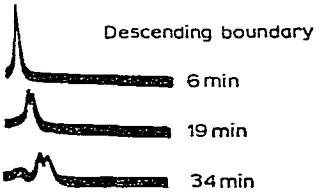


Fig. 9. Electrophoresis in borate buffer of fraction *B* (Fig. 7) of linear xylan from Townsville lucerne.

Interaction of hemicelluloses with metal ions. — The influence of the nitrate salts of a number of metals on linear xylan from Townsville lucerne in 20% aqueous ethanol was studied as follows. Redistilled ethanol (1 ml) was added, with efficient stirring, to a 0.5% aqueous solution of linear xylan (3 ml) in a centrifuge tube. An appropriate quantity of concentrated metal nitrate was added drop-wise by micropipette, together with the necessary complement of water to make a total addition of 1 ml. The solution was stirred for 15 min, placed in a bath at 30° for 1 h, and then centrifuged at 17,300 *g* for 20 min.

A portion of the centrifugate was analysed by the phenol–sulphuric acid procedure, and the percentage of xylan was calculated from a calibration curve. There was no time-dependence in the amount of material precipitated in the time range 1–24 h. The results are illustrated in Fig. 10.

DISCUSSION

In discussion the fractionation of plant hemicelluloses, we shall normally assume that the other major types of polysaccharide have previously been removed. This requirement is usually relatively easy to achieve, except in the case of pectic

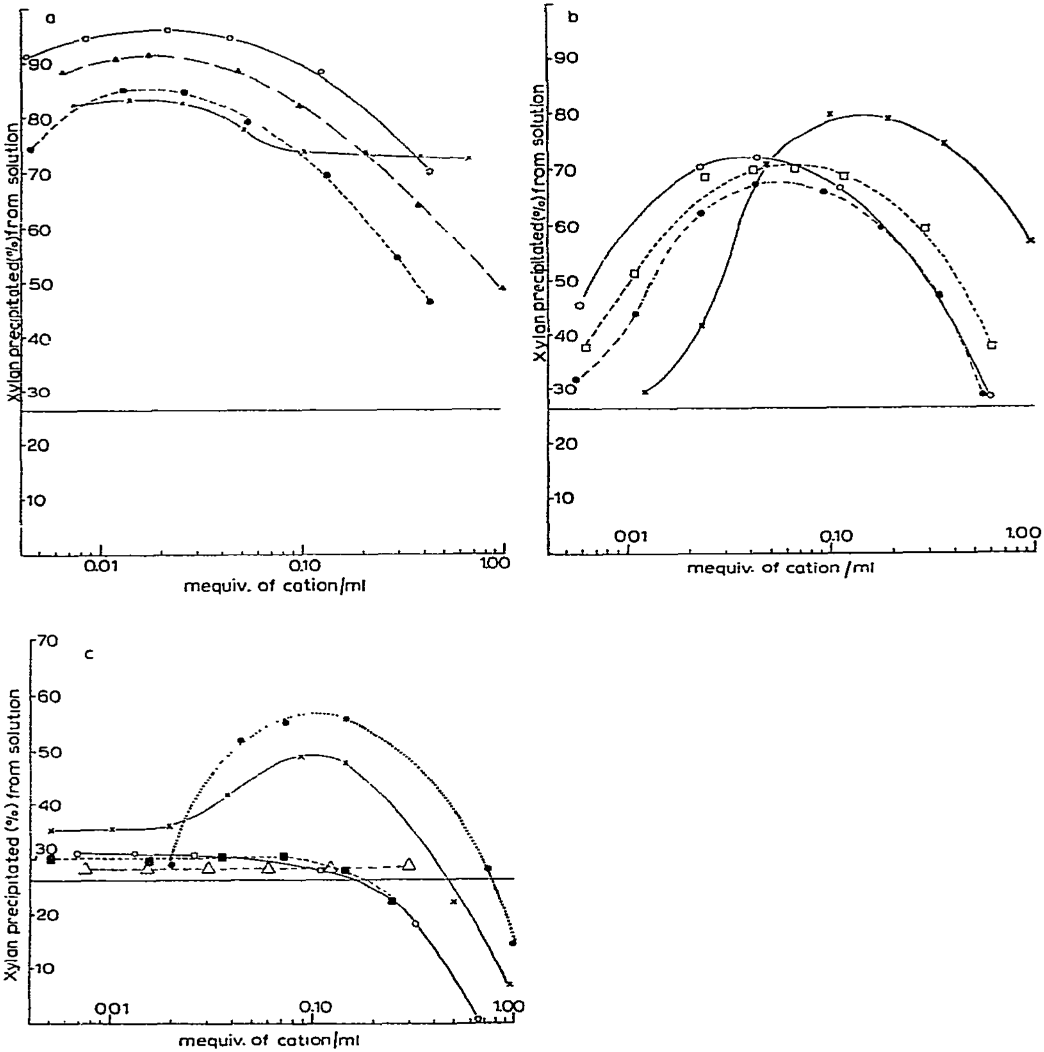


Fig. 10. Solubility curves in 20% ethanol for xylan from Townsville lucerne in the presence of various metal nitrates. (*a*) ○, lead; ▲, lanthanum; ×, aluminum; ●, copper. (*b*) ○, zinc; □, cobalt; ●, nickel; ×, silver. (*c*) ●, calcium; ×, magnesium; ○, sodium; ■, potassium; △, ammonium. Horizontal line, 20% ethanol.

substances which, if originally present in large proportion (as in pasture legumes), will frequently contaminate the hemicelluloses obtained by conventional procedures. In this context, we have found that iodine precipitation is useful as a preliminary fractionation tool, since the major, linear-xylan components are precipitated with iodine, leaving branched xylans and pectic substances in solution (*cf.* ref. 14). The major part of this study has therefore been carried out on this linear-xylan fraction which is essentially free from pectic substances.

Most known methods of polymer separation have previously been applied to hemicelluloses but without any generally applicable success. The effectiveness of fractional precipitation with ethanol has been demonstrated in particular instances by a number of workers[15–17]. Usually, however, the best separations are obtained with acidic polysaccharides under acidic conditions (pH 2–4), and the acid lability of L-arabinofuranosidic linkages, commonly found in plant xylans, requires some experimental caution. Thus, we have found that in fractional precipitations at pH 2, when the fractionation is not completed expeditiously (*e.g.*, if the solution is allowed to stand at room temperature for 4 h), free arabinose could be detected by paper chromatography of the mother liquor. The linear xylan from Townsville lucerne shows three components by inflection in the precipitation curve (Fig. 1) and by compositional analysis (Table I). However, the narrow range in ethanol concentration over which precipitation occurs virtually precluded large-scale application of this method of separation.

Chromatographic fractionation of polysaccharides on such materials as alumina, carbon, and synthetic ion-exchange resins has not proved very successful. Our results (Table II) show that irreversible sorption occurs, even in resins having a low degree of cross-linking, and confirm similar observations by Reid and Wilkie[18]. More success has followed the application of cellulose anion-exchangers for the fractionation of acidic polysaccharides. Neukom and Kuendig[19] suggested that these are readily sorbed on DEAE-cellulose at pH values near 6 and are eluted, depending on their carboxyl content, (*a*) by increasing the ionic strength of the displacing buffer, (*b*) by alkaline solutions of increasing strength, or (*c*) by acidic solutions of increasing strength.

Neukom and coworkers[20] claimed successful application of this approach in the fractionation of pectic substances and used sodium hydroxide to elute the main acidic polysaccharide in three qualitatively similar fractions. Rosik and co-workers[21] similarly separated a number of pectic substances into fractions which contained all of the monosaccharide components of the original mixture. The difficulty inherent in such application, however, lies in the use of alkali for displacement of substances known to possess linkages that are labile to alkali[22].

Our results also show other difficulties arising from the use of alkali for elution of polysaccharides, in that variable amounts of material giving a positive reaction with phenol–sulphuric acid were eluted from the column (Table II). These variations in recovery from the columns may be associated with variation in the rate of flow, although our evidence in this respect is incomplete. The recoveries, however, were often greater than 100%, and we conclude that carbohydrate-like material is eluted from the column packing by the alkaline eluant and that this elution would be more extensive when the column is run slowly for a given volume of eluent. This type of elution could not be prevented by prior reduction of the column packings with borohydride and showed no apparent diminution following prior treatment with 2M sodium hydroxide. Reid and Wilkie[23] hydrolysed these extractives and detected glucose which they concluded had arisen by "alkaline degradation of the ion exchanger".

It is difficult, however, to envisage any such rapid alkaline degradation occurring after our borohydride reduction of the ion exchanger, and yet we found that the prior reduction did not prevent this type of elution from the column packings. We prefer, therefore, to postulate a slow diffusion of relatively short polymer molecules from the packing. These short molecules were presumably produced during the original processing of the ion exchangers[24], and their diffusion into the eluant will be rate controlling and dependent on the extend of swelling of the particles.

Neukom and Kuendig[19] have shown that the structure of a polysaccharide strongly influences its adsorption on DEAE-cellulose. Their conclusions suggest that linear xylans would be expected to be strongly sorbed on cellulosic materials, and our experience (Fig. 5) demonstrates the ready binding of these materials. The mechanism is probably hydrogen bonding, and it is of interest to note that, although the xylan was almost completely removed from the solution by the cellulose, 39% could subsequently be eluted from the adsorbent with water. This is in keeping with variations noted in the passage of xylan solution through columns of cellulose, when the proportion retained was dependent on the amount of xylan applied. It would seem then that there are active sites in the cellulose which result in strong, irreversible sorption, and others capable of only loose association. No sorption was noted on cross-linked dextran, but this did not apply to the dextrans incorporating ion-exchange sites (Table II).

Jermyn[25] has reported the successful chromatography on DEAE-cellulose of a number of highly branched, acidic polysaccharides not associated in Nature with cellulose but found that hemicellulose from carnation-stem holocellulose could not be desorbed at high concentrations of eluting electrolyte. Aspinall and co-workers[26] noted irreversible sorption of xylan from soya beans on DEAE-cellulose and confirmed that a similar situation existed when the polysaccharide was passed through a column of cellulose.

Clayton and Phelps[27] studied in detail the sorption (in 20mM alkali) of glucomannan and xylan on α-cellulose from wood fibres and concluded that the sorption of both hemicelluloses is physical in nature. They ascribed the lower sorption of the xylan to the presence of carboxyl groups, (uronic acid) and concluded that a small percentage of these is apparently sufficient to override such factors as a lower degree of polymerisation and conformational similarity which would be anticipated to favour sorption of xylan on cellulose. It was suggested that the uronic acid content greatly influences the degree of hydration of the xylan, which in turn limits sorption.

DEAE-cellulose yielded a total recovery of 53% of a hemicellulose when displacement by an increasing concentration of acetic acid was attempted (Fig. 2.). A similar result was obtained with TEAE-cellulose (Fig. 3). A feature of this separation was the elution of a large portion of material coincident with a rapid decrease in in the pH of the column effluent. The earlier rise in pH may be associated with swelling of the cellulose fibres and sorption of hydrogen ions by exposed functional groups. After saturation of these groups, a sharply acidic pH-gradient could pass through the column as the effluent equilibrated to the acid concentration in the gradient. The severity of these pH changes on the column severely decreases the usefulness of this method of fractionation of acidic polysaccharides. The complex pH-profile in these experiments (and with QAE-Sephadex, Fig. 6) has also been noted on unloaded columns, and the only satisfactory method to date of establishing a linear pH-gradient has been the addition of "universal" type buffer (phosphate–citrate) with progressive diminution of its pH by an automated-titration device geared to the desired elution time for the experiment[28].

The manufacturers of DEAE-Sephadex report[12] that some difficulties arise in the introduction of diethylamino groups (p*K* 9.5) into the dextran matrix due to the simultaneous formation of a small amount of double groups

$$-CH_2CH_2\overset{+}{N}(C_2H_5)_2\ CH_2CH_2N(C_2H_5)_2.$$

The charge on the quaternised nitrogen atom impedes protonation of the tertiary nitrogen, and the p*K* of this group is 5.7. It follows that operation at nearly neutral pH will leave a significant percentage of these groups uncharged but this will diminish under the conditions of an acidic gradient. The titration curve for DEAE-cellulose (Fig. 4) gives evidence for groups of two different basicities and these may contribute to its observed behaviour with acidic gradients. However, no such evidence was obtained in the titration curve of TEAE cellulose (Fig. 4), and this type of explanation cannot be used to interpret the pH variations with columns of the quaternary amine resins (Figs. 3 and 6).

The "break-through" pH step in such gradient elution curves will normally coincide with an elution peak, and it is most unlikely that useful fractionation could occur in this region. This effect therefore imposes a significant limitation on the usefulness of such methods of fractionation.

Sorbed hemicellulose could not be eluted from QAE-Sephadex by using gradients of decreasing pH. However, the sorbed hemicelluloses were eluted, with successive batches of 0.5 and 2M sodium hydroxide in distinct peaks (*A*, *B*, *C* in Fig. 6), whose composition is shown in Table IV. The compositions suggest that the resolution into peaks *B* and *C* may be fortuitous, but the separation of peak *D* is evidently based on major, compositional differences, and this type of batch elution would normally be the method of choice for polymers known to be relatively stable in alkali.

The use of salt gradients for elution without concomitant changes in pH has also proved effective (Figs. 7 and 8). However, the use of cross-linked dextran derivatives, while superior to cellulose ion-exchangers in terms of reversible sorption characteristics for hemicelluloses, is complicated by bed-volume contraction arising from osmotic effects as the ionic strength increases. It is difficult to decide to what extent the observed fractionation arises from ionic displacement by the salt gradient or from physical contraction effects in the beads. However, this displacement by contraction of the column is probably of only secondary importance, since Table III shows that the proportion of xylan displaced increases rapidly up to 0.2M potassium chloride and slowly thereafter, whereas the contraction of the column continues to increase rapidly up to much higher concentrations of salt. Re-chromatography of fraction *B* eluted by the salt gradient (Fig. 7) revealed no significant amount of unretained fraction but decreasing recoveries indicated some irreversible sorption in each analysis. Furthermore, the demonstration of at least two components readily separated by electrophoresis (Fig. 9) in fraction *B* indicates a limited usefulness as a method of purification.

The conditions under which a particular molecule is eluted appear, in fact, to depend on unpredictable physical factors, which may include the contraction of the column packing during elution. Thus, for example, both peaks *B* and *D* were obtained when the original peak *B* was re-chromatographed (Fig. 7), and when peak *D* was re-chromatographed it was eluted early in the salt gradient, despite previously resisting elution with potassium chloride. Nevertheless, the method shows some potential usefulness in separation from mixtures of polysaccharides of the unretained fraction (*A*), which would normally be a non-acidic polysaccharide.

Variation in the amount of unretained material on QAE-Sephadex for the same xylan is of some concern. In this respect, loading capacity was not investigated, since the stated capacity of 3 mequiv./g seemed more than ample for the amounts of weakly acidic xylan used. However, it may be that the hydrodynamic requirements of these linear xylans allow only limited access to exchange sites situated in a cross-linked matrix.

Compositions of retained and unretained fractions (Table V) indicate that some purification can be obtained by passage through QAE-Sephadex, particularly in the removal of neutral glucan which may be cellulose of low molecular weight extracted with the hemicelluloses. However, it is noted that significant quantities of glucose remain in the retained polymer (peak *B*), and it is uncertain whether this arises through attachment to the xylan or from partially oxidised fragments of cellulose produced during delignification[29].

The interactions of metal ions with hemicelluloses are also of interest. In all cases studied, we found that small amounts significantly decrease the solubility of the polymer. The extent of the decrease in solubility is presumably associated with, and depends primarily upon, formation of a complex of the metal ions with hydroxyl groups of the polysaccharide, although some intermolecular, ionic bonding may also occur between polyvalent metal ions and the carboxyl groups of the uronic acid. However, all except ammonium ion also show a concentration beyond which the solubility of the complex increases significantly (Figs. 11*a*, *b*, *c*). Haug and Smidsrod[30], working mainly on polyuronides, have discussed these two types of salt effects. They also found that the addition of neutral salts to solutions of charged polysaccharides decreases the solubility of the polymers. The concentration necessary to cause salting-out depended on the ethanol concentration, type of cation present, and nature of the charged group and its stereochemistry. At salt concentrations above 0.2M, marked changes were noted in the solubility of the polysaccharide, either increasing or decreasing, depending on the nature and the type of neutral salt. They suggest that, since this second effect occurs at approximately the same salt concentration, independently of the ethanol concentration and independently of the presence and nature of charged groups in the polymer, it may be associated, in some way, with changes in water structure induced by the salts, and this postulate was supported by reference to the measurement by Frank and Wen[31] of entropy changes induced in water by various cations.

Our results, while demonstrating the effect, do not entirely support the observations of Haug and Smidsrod. Considerable variation occurs over the critical concentration range at which the salting-in effect occurs, and more-detailed study into the nature of the complexing and the influence on water structure is required before adequate interpretation can be made.

An important aspect of these results lies in the accepted use of metal complexes for fractionation of polysaccharides. Jones and Stoodley[32], in describing fractionation by copper complexing, noted that the usual procedure involves addition of excess precipitant, and Meier[33] advocates addition of reagent "until precipitation of barium complexes of mannan is complete". Such procedures, in the light of our results (Fig. 11), require modification and should be based on small-scale solubility curves; otherwise, it is evidently all too easy to add the salt solution to a position beyond the maxima shown in Fig. 11, with resultant "salting-in" of the precipitate.

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