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# Analysis of larch arabinogalactan by high performance size-exclusion chromatography

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The elution behaviour of larch arabinogalactan (AGal) during size-exclusion chromatography (SEC) in aqueous solutions, using a Separon S HEMA Bio 1000 column, has been investigated. It is established that the polydispersity of AGal in pure water is caused by its chemical heterogeneity. It is found that to obtain the correct molecular mass distribution (MMD) of AGal, SEC measurements should be performed with an 0·4 M acetate buffer of pH 5·5 as the eluent to prevent polyelectrolyte effects. From the SEC data the larch AGal is characterized by a nearly Gaussian MMD, a narrow polydispersity ($M_w/M_n \sim 1{\cdot}1$–$1{\cdot}4$) and an $\bar{M}_w$ of ca. 10 000.

## 1 INTRODUCTION

Arabinogalactan is a water-soluble polysaccharide naturally occurring in larch wood in considerable amounts (5–30%) (Kin, 1980). The molecular mass (MM) parameters of arabinogalactan have been studied by viscometry (Owens, 1940), osmometry (Husemann, 1940), ultracentrifugation (Mosimann & Svedberg, 1942), and gel-filtration (Ettling & Adams, 1968; Simson *et al.*, 1968). Today, high performance size-exclusion chromatography (HPSEC) is the most widely used technique to determine the molecular mass distribution (MMD) of polymers, including water-soluble polysaccharides (Barth, 1980; Barth & Smith, 1981). Nevertheless, there are no previous reports on HPSEC of arabinogalactan.

In the present work the effect of pH, salt concentration, and sample concentration on the elution behaviour of larch arabinogalactan is investigated using a Separon S HEMA Bio 1000 column.

## 2 EXPERIMENTAL

### 2.1 Materials

For isolation of arabinogalactan, extractive-free wood meal of Siberian larch (*Larix sibirica*) was used. The arabinogalactan was isolated by extraction with water for 72 h at room temperature, at a ratio of 1 part of meal to 25 parts of water. The arabinogalactan was precipitated in ethanol and then dried *in vacuo*, resulting in a cream-coloured powdered material (Bouveng & Lindberg, 1959).

The following dextran standards were obtained from Pharmacia (Fine Chemicals AB, Uppsala, Sweden): DT 10, DT 40, DT 70, DT110, DT 170, DT 500, and DT 2000. Polyethylene glycols PEG 300, 600, 1000, 3000, 6000, 15 000, 20 000, 40 000 and raffinose were purchased from Fluka (Buchs, Switzerland). Cellobiose and glucose were obtained from Sigma (St Louis, MO, USA).

### 2.2 Size-exclusion chromatography (SEC)

The analysis was carried out using a laboratory instrument work chromatograph GPC (Prague, Czechoslovakia) equipped with a refractive index detector and a Rheodyne 7125 injection valve. The prepacked column was of stainless steel (250 × 8 mm i.d.) containing 10 $\mu$m Separon S HEMA Bio 1000, obtained from TESSEK (Prague, Czechoslovakia). The mobile phase was distilled water and acetate buffer (0·2M sodium acetate and 0·2M acetic acid). The temperature was ambient and the flow-rate was 0·4 ml/min. The injection volume was 20–100 $\mu$l. The solutions were filtered through a 0·5 $\mu$m Millipore filter prior to use.

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## 3 RESULTS AND DISCUSSION

The chromatogram of arabinogalactan eluted with water indicates the multimodal distribution pattern, as shown in Fig. 1. The elution curve shows the presence of three fractions: fraction I (termed FI) eluting at the void volume ($V_0$) and fractions II (FII) and III (FIII) eluting somewhat later, at 4·8 and 5·9 ml, respectively. These results are similar to previously reported results for arabinogalactan where a bimodal MMD of larch arabinogalactan was suggested (Mosimann & Svedberg, 1942). Larch arabinogalactan was regarded as a mixture of two polysaccharides, one having an MM of 100 000–75 000 and the other having an MM of 16 000–14 000 (Mosimann & Svedberg, 1942; Simson *et al.*, 1968). Later work supported this interpretation (Salyers *et al.*, 1981).

However, the arabinogalactan is a polyelectrolyte as it contains galacturonic residues, and according to the literature (Barth, 1980; Barth & Smith, 1981), SEC of water-soluble ionic polysaccharides is complicated by aggregation and polyelectrolyte effects if pure water is used as the eluent. The latter is revealed by concentration effects.

In this connection we investigated the effect of sample concentration on the arabinogalactan elution behaviour. With decreasing concentration of solute in the injected volume, the retention volume ($V_e$) of FI and FII is decreased, while the position and elution profile of FIII is unchanged. This suggests that the polymers

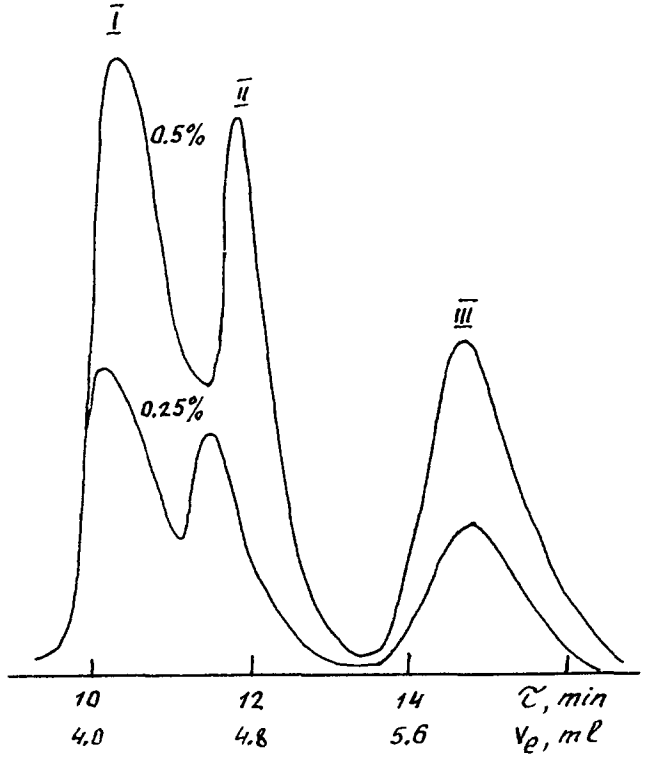


**Fig. 1.** The effect of sample concentration on arabinogalactan separation in water.

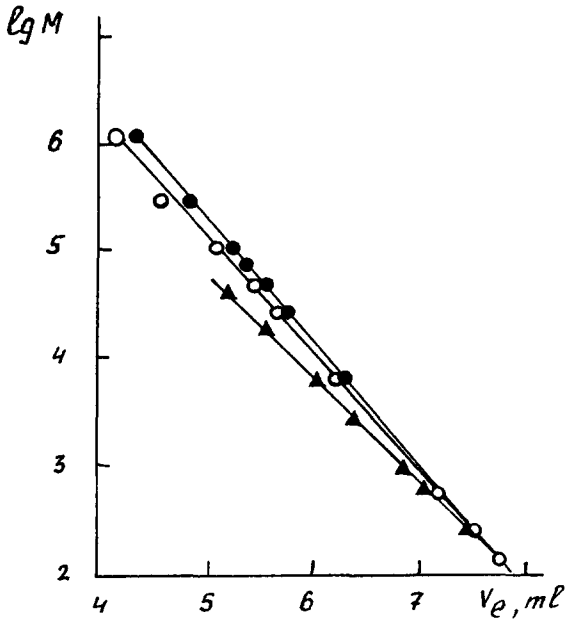


**Fig. 2.** Logarithm of molecular weight plotted against elution volume ($V_e$) for dextran in water (○) and in acetate buffer of pH 5·5 (●), and for PEG in acetate buffer of pH 5·5 (▲).

corresponding to FI and FII contain ionic groups that dissociate in water and therefore behave as polyelectrolytes, whereas FIII is practically neutral. It is logical to conclude that these fractions contain different amounts of galacturonic acid residues, and the separation of arabinogalactan in water is determined by its heterogeneity of chemical structure.

The formal estimation of arabinogalactan MM using dextran standards (Fig. 2) gives ca. 150 000, 70 000, and 10 000 for fractions I, II, and III, respectively. These MM values are in good agreement with the results obtained by the other investigators cited above. In our opinion the previous results reflect the different charge distributions of the polymer chains, but not the real MMD of this polymer.

The elimination of polyelectrolyte effects in SEC is possible by altering the ionic strength and/or pH of the mobile phase. For this purpose, the acetate or phosphate buffers are commonly used in SEC of charged water-soluble polysaccharides (Barth, 1980; Barth & Smith, 1981). In the present work acetate buffers with different pHs were used as the eluent.

As Fig. 3 shows, already at pH 5·5 the arabinogalactan elution curve is monomodal and no concentration dependence was observed. On further decreasing the pH, the chromatograms are practically identical. This shows that all polyelectrolyte effects are suppressed at pH 5·5. Thus, to obtain the correct MM parameters of arabinogalactan on Separon S HEMA Bio 1000, a pH of 5·5 is adequate.

The fractions comprising each peak eluted from the column with water were collected and refractionated with the acetate buffer as the eluent. As Fig. 3 illustrates,

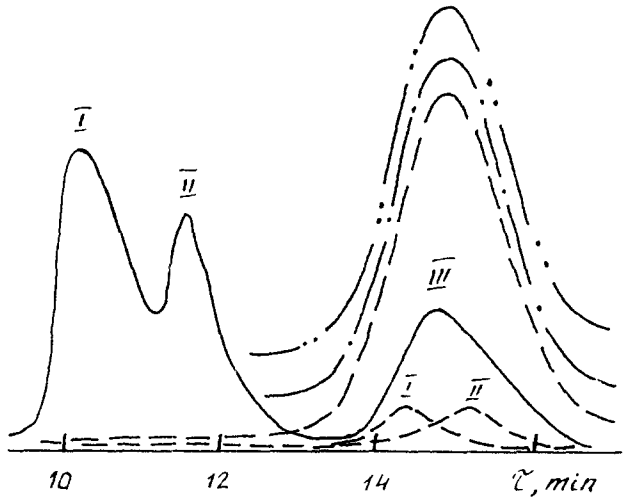


**Fig. 3.** The effect of ionic strength and pH of the mobile phase on arabinogalactan elution behaviour. Eluent: water (——) and 0·4M acetate buffer of pH 5·5 (– – –), pH 5·0 (–·–·) and pH 4·0 (–···–). the (– – –) profiles labelled I and II are the I and II fractions collected from the water eluted column and rechromatographed using pH 5·5 acetate buffer as the eluent.

they elute with the neutral FIII, and, moreover, the retention volume of FI is somewhat lower, but the $V_e$ of FII is somewhat higher than the $V_e$ of FIII. Such behaviour once more confirms that the arabinogalactan separation in water is dominated by its chemical composition and not MM.

The next step in our investigation was to determine the MM parameters of larch arabinogalactan. To estimate the dependence of MM on $V_e$ the chromatography system was calibrated with dextran standards and PEG. The calibration curves are linear over a wide range from $10^2$ to $10^6$ Da, as shown in Fig. 2.

Unfortunately, the Mark-Houwink constants for arabinogalactan in acetate buffer are not reported in the literature; therefore, the universal calibration concept cannot be applied. However, taking into account the fact that the constants estimated for PEG and other native polysaccharides in aqueous solutions are similar (Anger & Berth, 1986; Dawkins *et al.*, 1990), i.e. the hydrodynamic volumes of the macromolecules are comparable under these conditions, it is possible to calculate the arabinogalactan MM parameters by PEG calibration. The larch arabinogalactan MM values obtained were as follows: $\bar{M}_w$ = 9390 and $\bar{M}_n$ = 6890. These data seem to approximate to the real MM and, therefore, $\bar{M}_w$ does not exceed 10 000. Our work suggests that this polymer is characterized by a narrow polydispersity, $M_w/M_n$ = 1·36, and a nearly symmetric MMD.

## 4 CONCLUSION

The mechanism of arabinogalactan separation in SEC using water as the eluent is determined by its chemical heterogeneity. This makes it possible to quantify the arabinogalactan chemical composition.

To eliminate the polyelectrolyte effects on a Separon S HEMA Bio 1000 column with arabinogalactan, the 0·4M acetate buffer of pH 5·5 is required.

The larch arabinogalactan has a monomodal Gaussian MMD with a polydispersity of 1·3–1·4 and MM of ca. 10 000,

Finally, it should be noticed that the use of the Separon S HEMA Bio 1000 column provides well-resolved and reproducible arabinogalactan chromatograms in less than 20 min.

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