SHORT COMMUNICATION

CHROMATOGRAPHY OF ACIDIC POLYSACCHARIDES ON DEAE-CELLULOSE*

By M. A. JERMYN†

The chromatography of polysaccharides on resinous ion-exchangers has been reported by Steiner, Neukom, and Deuel (1958). Neukom *et al.* (1960) later reported the chromatography of a variety of wheat and sugar-beet polysaccharides on DEAE-cellulose. Only "neutral" polysaccharides could be eluted at pH 6–8 by increasing buffer and salt concentrations; acid polysaccharides, e.g. pectic acid, were eluted by various strengths of alkali. Neutral polysaccharides could also be chromatographed at a somewhat alkaline pH by working with borate systems, which give charged complexes with many carbohydrates.

Since DEAE-cellulose will be completely discharged in the alkaline range (0·01–0·5N NaOH) used by Neukom *et al.*, and acid polysaccharides completely ionized, the chromatographic results of these workers would appear to depend on adsorption rather than ionic interactions. The more logical alternative of working at a pH around 4, where DEAE-cellulose will be completely ionized and the carboxyl groups of acid polysaccharides partly and perhaps selectively discharged, does not seem to have been reported previously. This work was initiated by the fact that observed fractionations of the crude β-glucosidase of *Stachybotrys atra* in this pH range appear to be those of the polysaccharides that it contains.

Methods

The column used contained 10 g of the coarsest fraction of DEAE-cellulose powder (Eastman Corporation) that had been well washed with NaCl and NaOH solutions to remove impurities. No matter how well they have been washed, a small amount of carbohydrate is released from all DEAE-cellulose samples studied in this laboratory whenever a salt gradient is applied to them afresh; this has always to be allowed for in quantitative experiments and in fact makes quantitative estimation of small amounts of external carbohydrate experimentally bound to and eluted from these columns very uncertain. The column was 25 cm long and 2 cm in internal diameter; its hold-up volume was about 70 ml.

The supporting buffer used throughout was 0·02M sodium acetate, pH 4·1, and the column was cooled by circulating ice-water in a jacket. The latter condition was maintained simply to keep continuity with the enzyme work and there was no evidence that changes in temperature up to 25°C made any qualitative difference to the results.

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† Division of Protein Chemistry, C.S.I.R.O. Wool Research Laboratories, Parkville, Vic.

The polysaccharide sample (50 mg in 2 ml) was washed through the column with 250 ml of buffer and a sodium chloride concentration gradient then run through the column. The shape of this gradient is indicated in Figure 1 and was constant throughout all experiments. The effluent was collected in 10-ml fractions.

Carbohydrate was determined by the anthrone method (Jermyn 1956).

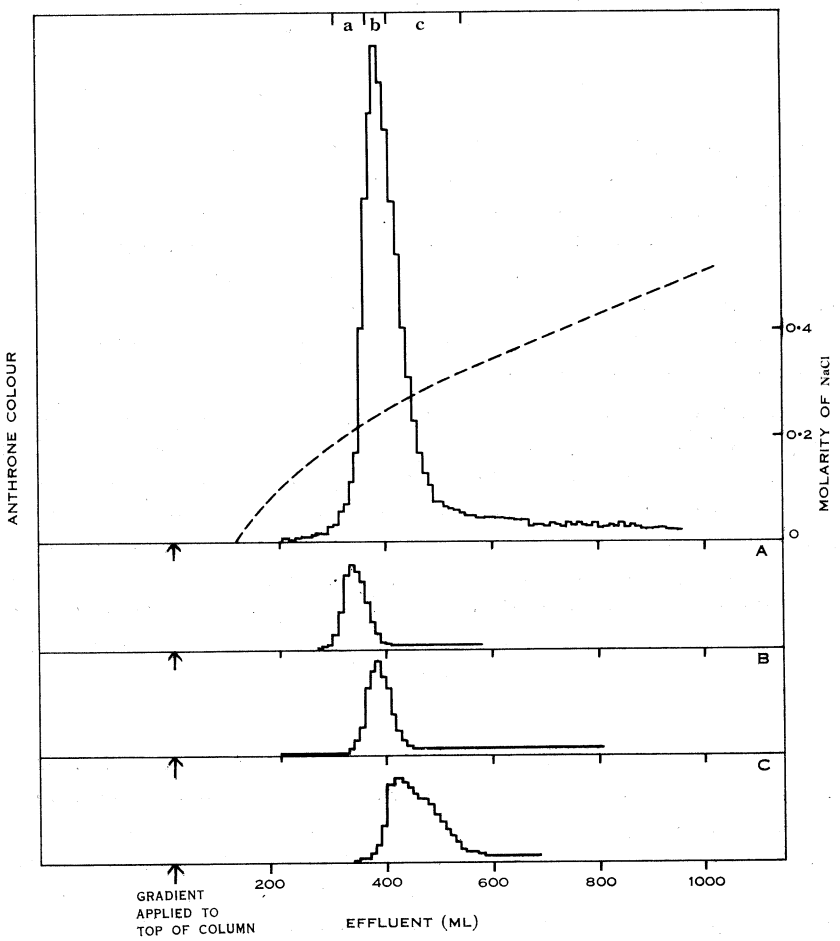

Fig. 1.—Elution curve for gum arabic (50 mg) from DEAE-cellulose (10 g) column at pH 4·1 in 0·02M sodium acetate buffer when the sodium chloride concentration gradient shown (– – – –) was used. *A*, rechromatography of material from *a*; *B*, rechromatography of material from *b*; *C*, rechromatography of material from *c*.

Results

(i) *Gum Arabic.*—Commercial gum arabic (a single tear and hence presumably the homogeneous product of a single plant) was dissolved in water. The result is shown in Figure 1.

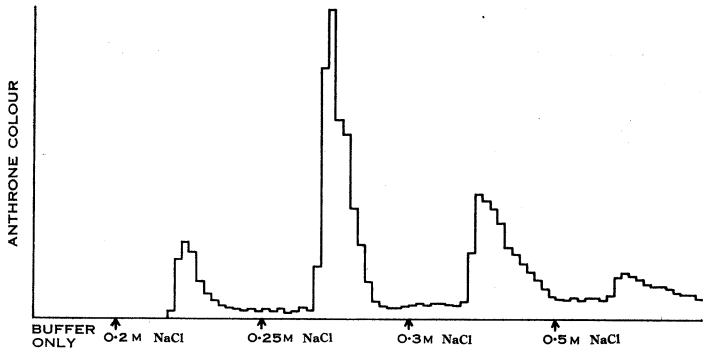

Fig. 2.—Stepwise elution of gum arabic (50 mg) under standard conditions. Eluate was 250 ml at each step.

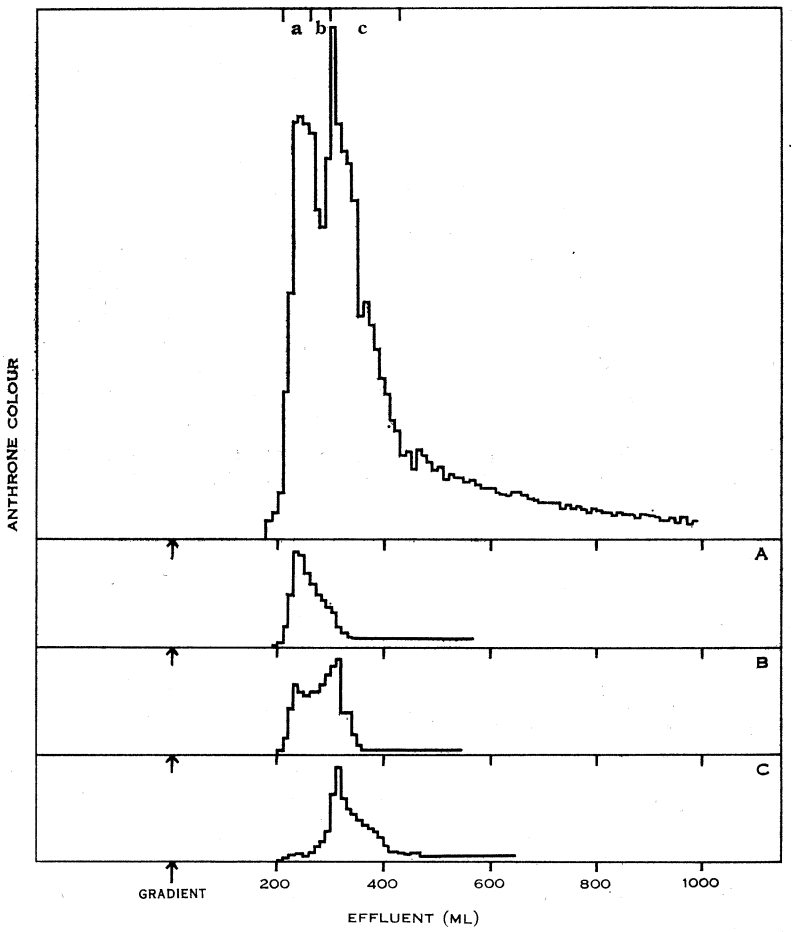

Fig. 3.—Elution curve for gum ghatti (50 mg) under standard conditions. *A*, rechromatography of material from *a*; *B*, rechromatography of material from *b*; *C*, rechromatography of material from *c*.

The appearance of a single peak in gradient elution (Fig. 1) is no guarantee that a single molecular species is being eluted; the most that can be said is that there is no sharp discontinuity in properties. The experiments in rechromatography illustrated in Figure 1, curves A, B, and C, incidentally indicate quite well how carbohydrate material eluted from the column by the gradient blurs the base-line against which the added polysaccharide is to be estimated. The relative inefficiency of gradient elution as a test of heterogeneity compared with stepwise elution is fully discussed by Tiselius, Hjerten, and Levin (1956).

An experiment in which the same quantity of gum arabic was eluted stepwise from the column under otherwise identical conditions is presented in Figure 2. The

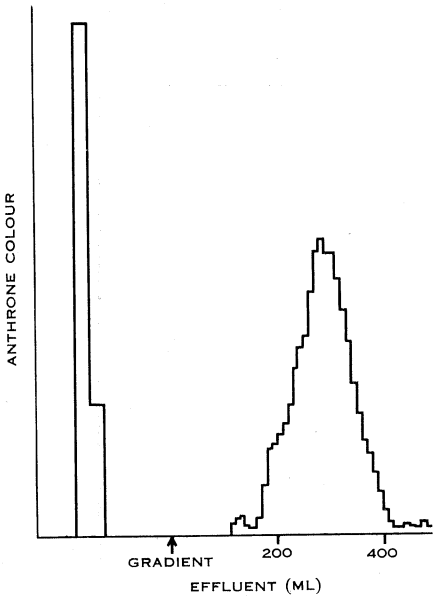

Fig. 4

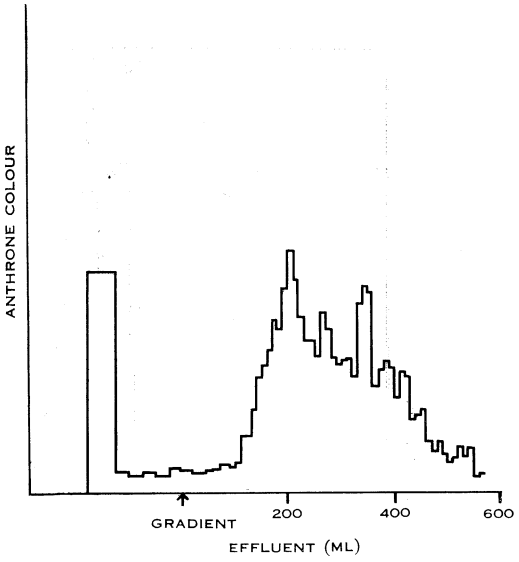

Fig. 5

Fig. 4.—Elution curve for wattle gum (50 mg) under standard conditions.

Fig. 5.—Elution curve for *Phalaris* polysaccharide (50 mg) under standard conditions.

results demonstrate even more clearly the "heterogeneity" deduced from the gradient-elution experiment, but it cannot be said whether this heterogeneity is physical or chemical.

(ii) *Gum Ghatti.*—A single tear of gum ghatti was ground and the powder suspended in water. The suspension was autoclaved for 60 min at 110°C to render the polysaccharide water-soluble, and the resulting solution centrifuged to remove a little insoluble gel. The results are shown in Figure 3.

The form of the elution curves and the results of rechromatography suggest that gum ghatti solutions as prepared contain two discontinuous series of related polymers.

(iii) *Wattle Gum.*—A sample of wattle gum has been prepared by Dr. G. Youatt (to whom acknowledgment for the gift of samples is made) from the exudate of a single tree of black wattle (*Acacia mollissima*). The gum was purified by several reprecipitations from slightly alkaline aqueous solutions with acidified ethanol. This material was dissolved in a little very dilute NaOH and the solution brought to pH 4·1 with dilute acetic acid. The results are shown in Figure 4.

Wattle gum appears to contain one polysaccharide fraction uncharged at pH 4·1, or at least not sufficiently charged to be retained on the column. The marked asymmetry in the main elution curve suggests that more than one polymer series is present.

(iv) Phalaris *Polysaccharides.*—During the chlorite delignification of cell wall material from the grass *Phalaris tuberosa*, Dr. Youatt found that water-soluble polysaccharide was liberated into the solution. This material would be expected to be somewhat degraded and to contain not only its original uronic acid carboxyl groups but also additional ones formed by oxidation. The polysaccharide was dissolved in dilute NaOH and the solution brought to pH 4·1 with acetic acid; the results are shown in Figure 5.

The elution curve corresponds with what would be expected for an exceedingly heterogeneous mixture, without much common ground plan in the polymers involved.

(v) *Carnation Hemicellulose.*—From the alkaline extract of carnation stem holocellulose, Dr. Youatt prepared a hemicellulose containing xylose and a little uronic acid. Like his other samples it would dissolve completely in very dilute NaOH to give a solution that remained stable indefinitely when adjusted to pH 4·1 with acetic acid. However, when such a solution containing 50 mg of hemicellulose was applied to the column, the sodium chloride gradient failed to elute the polysaccharide. Nor did subsequent passage of stronger sodium chloride solutions (up to 5M) dislodge it. It is to be concluded that at pH 4·1 carnation hemicellulose was irreversibly bound to DEAE-cellulose. This type of chromatography therefore appears to be most satisfactory for highly branched polysaccharides not associated with cellulose in nature (e.g. gum arabic). It is unsuitable for relatively linear polysaccharides occurring naturally in association with cellulose (e.g. carnation hemicellulose).

References

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