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Note

Convenient, in-line purification of saccharide mixtures in automated high-performance liquid chromatography

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Precolumns have been applied effectively to all types of liquid chromatography for years. Specifically, in liquid–liquid chromatography, precolumns have been used to condition the eluent and prevent stripping of the stationary phase[1]. In addition, precolumns have been used to remove and concentrate components from complex mixtures while unwanted components were discarded[2] or alternately to retain on a column unwanted components which interfere with the final chromatogram[3]. More recently, chemical modification of specific components in a mixture using a derivatizing precolumn has been introduced in high-performance liquid chromatography (HPLC)[4,5]. A mixed bed de-ionizing precolumn has been suggested[6] but peak broadening was reported as a difficulty.

The analysis of corn syrups and sugar mixtures (Ca^{2+}-form of cation-exchange resin with water as eluent) using HPLC equipped with a refractive index detector[7], is complicated when inorganic salts (hereafter referred to as ash) are present. The ash elutes with higher saccharides causing incorrect results. Ash is removed either by batch ion-exchanging, with mixed resins or by layered resins in small ion-exchange columns. With the recent development of automated sampling devices and resulting high sample throughput, the cumbersome and time consuming manual de-ashing (de-ionizing) of many samples becomes a limiting factor. An inexpensive, efficient, in-line de-ashing precolumn is needed that does not produce peak broadening and loss of resolution in the final chromatogram.

EXPERIMENTAL

A piece (10 cm × 3 mm I.D.) of stainless-steel tubing (Alltech, Arlington Heights, Ill., U.S.A., Cat. No. 3013) was fitted with two 1/4 in. end fittings, nuts and ferrules. The end fittings (Waters Assoc., Milford, Mass., U.S.A., Cat. No. 27476) were supplied with 5 μm frits. Teflon washers (Waters Assoc., Cat. No. 27495) were used in the end fittings but could be eliminated at the discretion of the chromatographer. Fig. 1 is a pictorial view of the precolumn.

The bottom half of the column was packed with a weak anion-exchange resin (OH^- form of Bio-Rad AG 3, 200–400 mesh, Bio-Rad Cat. No. 140-4351, Bio-Rad Labs., Richmond, Calif., U.S.A.). Subsequently, the top was packed with a strong

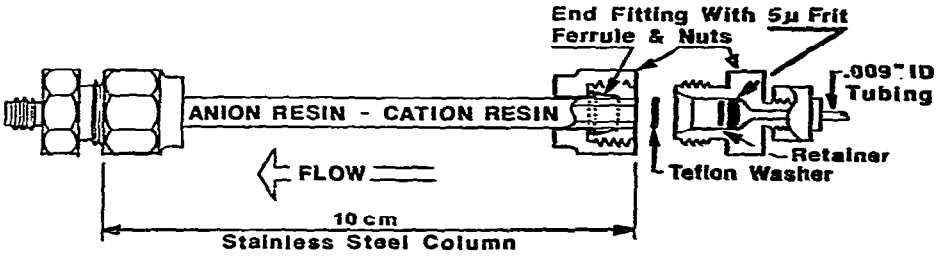

Fig. 1. Construction of column assembly.

cation-exchange resin (H^+ form of Bio-Rad Q-150S 21–35 μm, Bio-Rad Cat. No. 147-2103). Both resins were packed using conventional slurry packing techniques.

Resin selection is an important factor in precolumn applications since loss of resolution could result from improper particle size. Small controlled particle size resins are generally preferred over gross mesh sized resin because superior resolution can be achieved. The Q-150S cation resin was selected because of low cost and had a controlled particle size distribution. The AG 3 weak anion resin was used for lack of a commercially available resin of this type, in controlled particle sizes.

Column size was balanced against resin capacity to accommodate approxi-

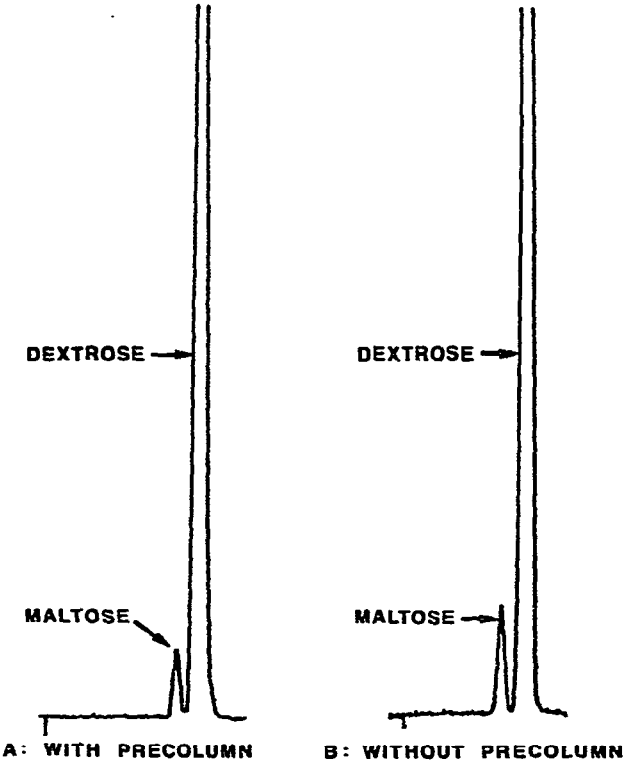

Fig. 2. Precolumn effect on resolution.

mately 1600 samples (20 μl of 10% syrup solution containing 0.5% dry basis ash) or about 6 weeks of operation. The actual number of samples accommodated in use depends on the ash levels of the samples.

For evaluation the column was attached [via 1 in. of 1/16 in. (0.009 in. I.D.) tubing, nuts, and ferrules] to an analytical column (61 cm × 7 mm I.D. packed with Bio-Rad Q-15S in the calcium form) installed in a Waters Assoc. ALC 201 liquid chromatograph.

RESULTS AND DISCUSSION

Fig. 2 shows a typical dextrose–maltose solution (97.12:2.88, w/w) examined with and without the de-ashing precolumn. No significant loss of resolution was observed with the addition of the precolumn. The chromatograms in Fig. 3 demonstrate the effectiveness of the precolumn.

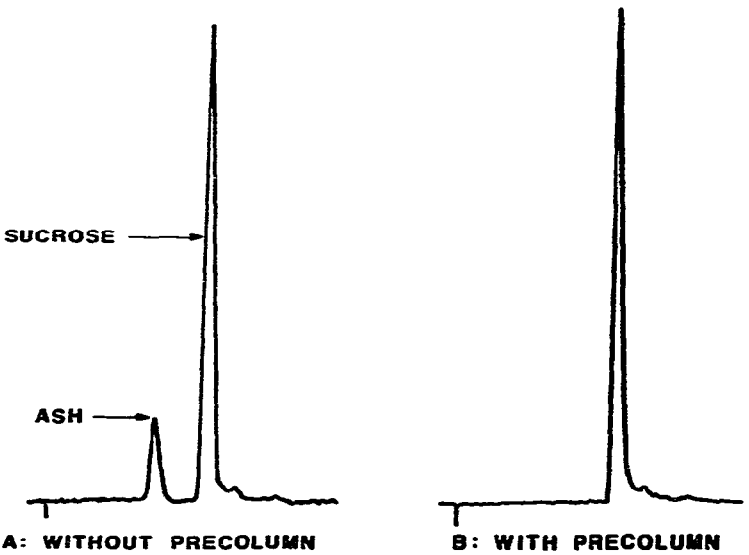

Fig. 3. Effect of ash removal on chromatogram.

The system with the precolumn was operated for a three-month trial period, during which 2300 samples were analyzed without exceeding the capacity of the resins.

To monitor the performance of the precolumn, a quality control sample with known ash content was analyzed daily. A sucrose solution containing sodium chloride (1.5 g of sucrose + 0.1 g of NaCl per 100 ml of H_2O) serves a dual purpose. The primary function is to monitor the efficiency of the precolumn to remove ash. A secondary role, performed by the sucrose, is to check the quality of the analytical column. For example, when H^+ ions replace Ca^{2+} ions on the resin, sucrose is inverted to glucose and fructose (Fig. 4A, B).

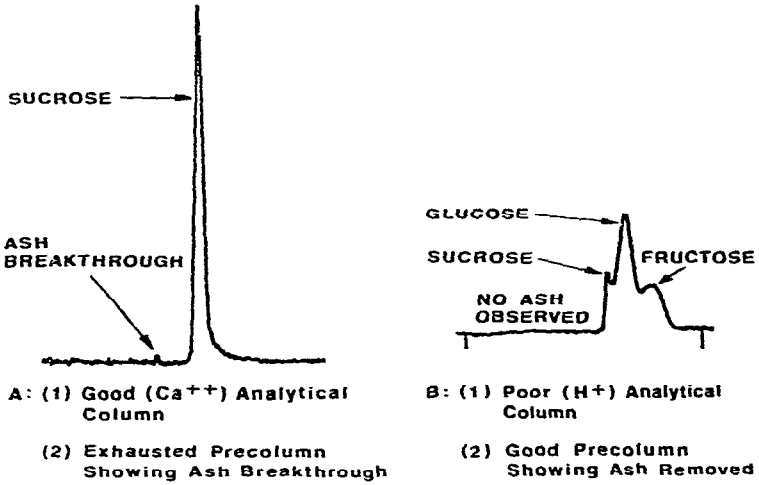

Fig. 4. Utility of sucrose-ash quality control solution.

CONCLUSION

A de-ashing precolumn has proven to be an inexpensive, efficient, automatic means of removing interfering inorganic salts from corn syrup and sugar samples. The precolumn permits the more efficient use of new automatic sampling systems by eliminating the operator time spent for manual de-ashing procedures.

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