Journal of Chromatography, 117 (1976) 206–210

CHROM. 8740

Note

A chemically bonded stationary phase for carbohydrate analysis in liquid chromatography

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(First received August 20th, 1975; revised manuscript received September 12th, 1975)

Progress in high-speed liquid chromatography in the last few years has been due largely to improvements in column technology. In this paper, we describe a simple and inexpensive procedure for bonding an aminoalkyl substituent to the surface of silica gel and discuss the separations of carbohydrates on columns packed with this material.

Aminoalkyl groups were chosen as chemically bonded stationary phase as amino-substituted anion-exchange resins have been successfully applied to the separation of saccharides. Silica gel as a support has the advantage of being pressure stable and readily available in suitable particle sizes and many different qualities.

Although carbohydrate separations similar to those we obtained with our packing material have been previously reported, *e.g.*, by Palmer[1], Linden and Lawhead[2] and many others, neither the chemical nature of the bonded stationary phase used nor the preparation of the packings were discussed.

EXPERIMENTAL

Equipment

The liquid chromatographic system was home made from commercially available components. The solvent was contained in a reservoir and stirred with a magnetic stirrer at room temperature to provide continous degassing. A Waters Model 6000 pump (Waters Assoc., Milford, Mass., U.S.A.) was used to deliver the solvent to the top of the glass column (250 mm × 3 mm I.D.). The chromatographic column was a high-precision KPG-tube (Jenaer Glaswerke, Mainz, G.F.R.) made of Duran 50 glass (Gebr. Möller, Zurich, Switzerland).

Injections were made by syringe (Precision Sampling Corp., Baton Rouge, La., U.S.A., Cat. No. 18001) through an LC inlet (Precision Sampling Corp., Cat. No. 420144).

An RI detector (LDC 1107, Laboratory Data Control, Riviera Beach, Fla., U.S.A.) was used to monitor the eluent. The chromatograms were recorded on a W+W 1100 recorder (Brechbühler AG, Urdorf, Switzerland).

Materials

The silica gel used to prepare the packing material was Lichrosphere SI 100,

a spherical silica with a particle diameter of 5 μm and an average pore diameter of 100 Å (E. Merck, Darmstadt, G.F.R., Cat. No. 9316). Prior to the bonding reaction, the silica gel was conditioned to a relative humidity of 15% by placing it over a saturated solution of lithium chloride in a desiccator.

3-Aminopropyltriethoxysilane was obtained from Aldrich (Milwaukee, Wisc., U.S.A., Cat. No. 11.339-5) and used as received. Toluene was of analytical-reagent grade, obtained from Merck (Cat. No. 8325). It was stored over molecular sieves. Acetone (Merck) and methanol (Fluka, Buchs, Switzerland) were of analytical-reagent grade and were used without further purification. Acetonitrile was obtained from Fluka (purum, Cat. No. 743222), filtered through acidic and basic alumina (Woelm, Eschwege, G.F.R.) and distilled. The water used in the mobile phase was twice-distilled.

Preparation of the packing

The chemically bonded stationary phase was prepared in the following manner. A 5-g amount of Lichrosphere SI 100 was placed in a 250-ml round-bottomed flask and 100 ml of a 5% solution of aminopropyltriethoxysilane in toluene were added. The slurry was degassed by evacuating the reaction vessel. After 15 min, the vacuum was replaced with nitrogen and the flask was immersed in a water-bath at 90° for 5 h.

After the reaction, the slurry was cooled to room temperature, filtered over a glass frit and washed at least five times with toluene. The packing was then rinsed with acetone, methanol and more acetone. The packing was then dried at room temperature by passing dry nitrogen through it.

Preparation of the column

A high-pressure slurry packing technique was used to pack the glass column. In order not to expose the glass tubes to the high filling pressure, the packing was carried out in a pressure vessel in which the pressure outside the column was equal to the filling pressure.

A 1.5-g amount of packing was suspended in 5 ml of methanol and the slurry was placed in the reservoir and forced into the column. Usually, pumping for 2 h at 350 atm was sufficient.

RESULTS AND DISCUSSION

Fig. 1 shows the separation of ribose, rhamnose, xylose, fructose and glucose. By increasing the acetonitrile content of the mobile phase, the resolution can be brought to almost any desired value.

Fig. 2 shows the separation of ribose, arabinose, glucose, saccharose, maltose, lactose and maltotriose. As a further example for the application of such separations, Fig. 3 shows the chromatogram of Maltrin 10, a soluble starch powder.

A significant characteristic of this bonded phase is its versatility. With the same phase system, not only neutral sugars, but also amino sugars and poly hydric alcohols can be separated. Fig. 4 shows the separation of 1-aminoxylose, 1-aminomaltose and 1-aminolactose. By reducing the water content of the mobile phase, the amino sugars can also be separated from their corresponding neutral sugars. An example of a

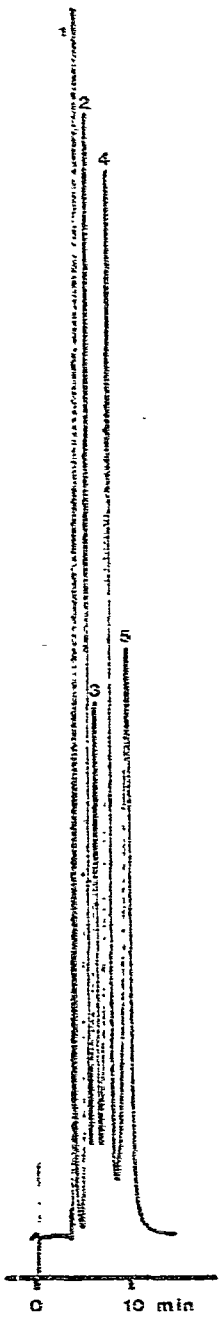

Fig. 1. Separation using 15% water–acetonitrile as mobile phase at 0.5 ml/min. Peaks: 1 = ribose; 2 = rhamnose; 3 = xylose; 4 = fructose; 5 = glucose.

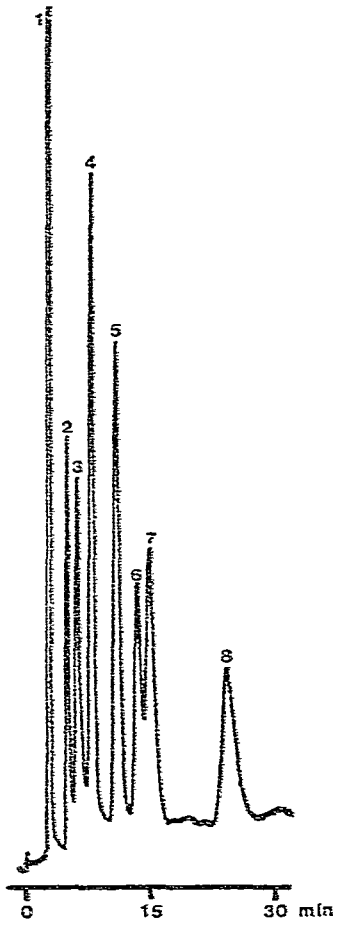

Fig. 2. Separation using 20% water–acetonitrile as mobile phase at 0.5 ml/min. Peaks: 1 = water; 2 = ribose; 3 = arabinose; 4 = glucose; 5 = saccharose; 6 = maltose; 7 = lactose; 8 = maltotriose.

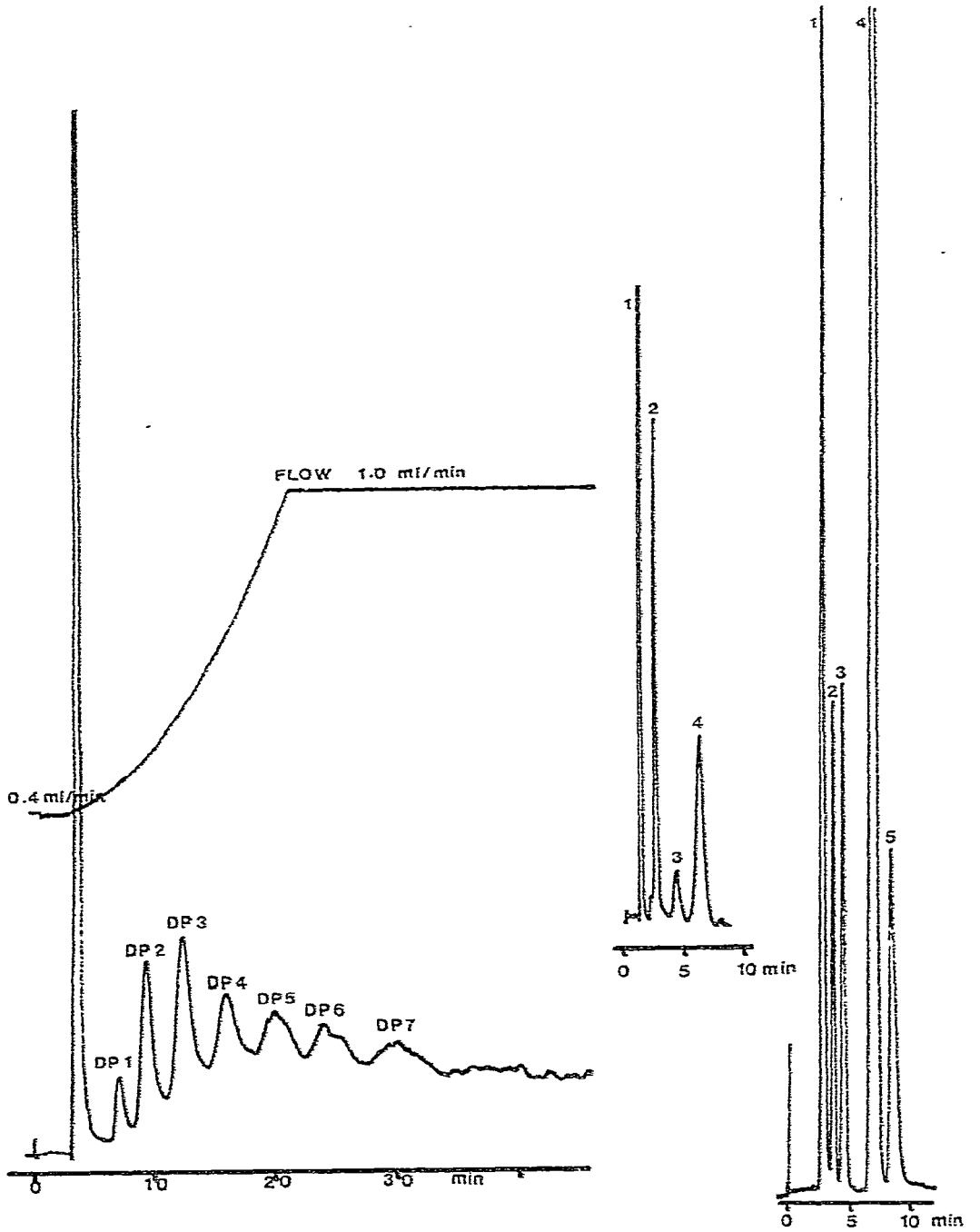

Fig. 3. Chromatogram of Maltrin 10 (soluble starch) obtained using 25% water–acetonitrile as mobile phase with a flow programme. DP1–DP7 = dextrose polymers.
Fig. 4. Separation using 20% water–acetonitrile as mobile phase at 1.0 ml/min. Peaks: 1 = water; 2 = 1-aminoxylose; 3 = 1-aminomaltose; 4 = 1-aminolactose.
Fig. 5. Separation using 20% water–acetonitrile as mobile phase at 0.5 ml/min. Peaks: 1 = water; 2 = ethylene glycol; 3 = glycerol; 4 = xylitol; 5 = sorbitol.

separation of polyhydric alcohols is shown in Fig. 5. Ethylene glycol, glycerol, xylitol and sorbitol were well separated from each other.

The bonding of an alkoxysilane to silica gel as described above is a polymerization rather than a substitution reaction. The amount of surface water on the silica gel determines the degree of polymerization. As the aminopropyltriethoxysilane contains three reactive sites, cross-linking or/and linear polymerization reactions may occur. The extent of these reactions is difficult to control[3].

A thick layer of bonded phase may lead to a low chromatographic efficiency owing to slow solute diffusion. A thin layer, on the other hand, may result in a low sample capacity and peak tailing. Investigations into the exact nature of this chemical bonding have not yet been made; the phase does, however, provide an excellent means of separating carbohydrates.

REFERENCES

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