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Note

Separation of sugars by h.p.l.c. on copper silicate gel

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Stationary phases most frequently used in h.p.l.c. of sugars involve silica gel and, particularly, alkylamine-bonded silica gel[1]. Usually, the mobile phase consists of water–acetonitrile mixtures; for silica gel, a polyfunctional amine is added[2].

We now report on the behaviour of sugars on a new stationary phase comprising a copper(II) silicate gel which has already been used for amino acids and

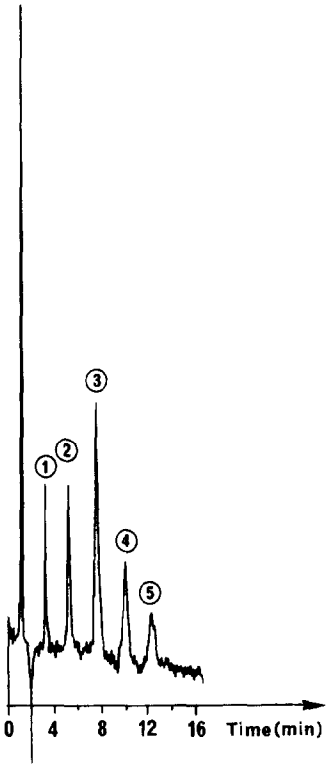

Fig. 1. Separation of 1, D-xylose; 2, D-glucose; 3, sucrose; 4, maltose; and 5, lactose on a column (150 × 4.8 mm i.d.) of copper silicate gel, using water–acetonitrile (25:75, $[NH_3]$ = 0.46M) at 2 mL/min at 30° and detection with a differential refractometer.

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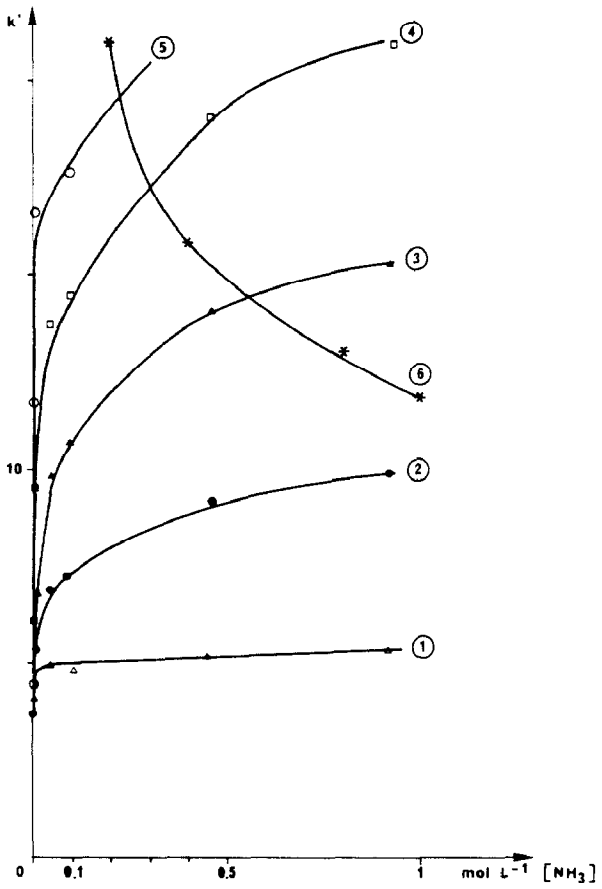

Fig. 2. Variations in the capacity factor k′ as a function of the concentration of ammonia in water–acetonitrile for 1, D-xylose; 2, D-glucose; 3, sucrose; 4, maltose; 5, lactose; and 6, 2-amino-2-deoxy-D-glucopyranose. Conditions as in Fig. 1.

peptides[3–6]. Of the original hydrogen atoms in the silanol groups, ~40% are substituted by copper(II). In the presence of ammonia in the mobile phase, the copper(II) silicate structure may be described as $(\equiv Si\text{-}O)_2Cu(NH_3)_x,(H_2O)_y$, with $1 \leqslant x \leqslant 2$.

Usually, the mobile phase consists of water–acetonitrile–ammonia mixtures, and the retention is generally explained[7] by (*a*) ligand exchange involving complexes between the solutes and copper(II) for electronic doublet donor compounds, and (*b*) normal phase partition between the mobile phase and water molecules solvating ammonia coordinated to copper(II); the most hydrophilic components have the higher retention times. For sugars which do not give stable complexes with copper(II), the retention is believed to involve the partition mechanism.

A typical separation of sugars on copper silicate gel is presented in Fig. 1. The capacity factor k′ for the sugars decreased when the water content of the

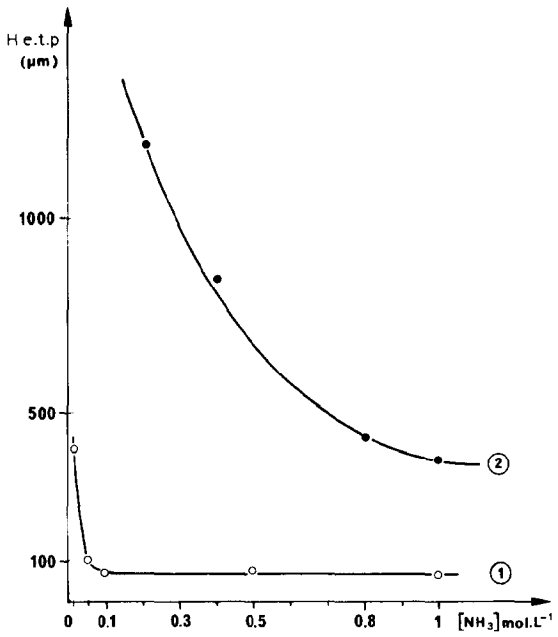

Fig. 3. Variations in h.e.t.p. for 1, D-glucose; and 2, 2-amino-2-deoxy-D-glucopyranose; as a function of the concentration of ammonia in water–acetonitrile (40:60) at 25°. Other conditions as in Fig. 1.

mobile phase increased, the ammonia concentration being kept constant. This observation accords with a normal partition mechanism. The variation in k′ for five sugars and one amino sugar (2-amino-2-deoxy-D-glucopyranose) with variation of the concentration of ammonia in the mobile phase is shown in Fig. 2. From these results, it is concluded that the retention of 2-amino-2-deoxy-D-glucopyranose depends on a ligand-exchange mechanism, whereas that of a neutral sugar is based on partition phenomena between the water of solvation of the ammonia molecules contained in the stationary phase and water in the mobile phase. These results accord with those observed with alkylamine-bonded silica gel. Verhaar and Kuster[8] suggested that the similar behaviour of alkylamine-bonded silica and ion-exchange resins in the retention of sugars reflects a partition mechanism between water of solvation of the amino groups and the hydro-organic mobile phase. Thus, the chromatography characteristic of sugars on silica may be similar to those observed by Samuelson[9] on ion-exchange resins using water–ethanol mixtures as mobile phases. This author has shown that the stationary liquid in the ion-exchanger was richer in water than the mobile phase, and a partition mechanism between the liquid phase immobilised into the ion-exchanger pores and the mobile phase was inferred.

The stationary phases aminopropyl-bonded silica gel, copper silicate gel in the presence of ammonia, and ion-exchange resins each have an affinity for water which results in the formation of a liquid stationary film rich in water. Copper

silicate gel has a high level of water solvation of the ammonia molecules coordinated to the copper(II).

For 2-amino-2-deoxy-D-glucopyranose (curve 6, Fig. 2), the retention time is affected by complex formation between the solute and the copper(II), mainly by a ligand-exchange mechanism. There is a competition between 2-amino-2-deoxy-D-glucopyranose and ammonia molecules, and the higher the concentration of ammonia in the mobile phase the lower is the retention time of the solute.

Fig. 3. compares the height equivalent to a theoretical plate (h.e.t.p.) for a column of copper silicate gel measured on the elution peaks of D-glucose and 2-amino-2-deoxy-D-glucopyranose. The h.e.p.t. for D-glucose is constant for $[NH_3] \geq 0.1$M, but decreases markedly for 2-amino-2-deoxy-D-glucopyranose. A similar observation was made in the separation of amino acids and is general for electronic doublet donor solutes. For a partition mechanism, the concentration of the ammonia has no influence on the efficiency of the column which is always greater than for a ligand-exchange mechanism.

The advantages of copper silicate gel over alkylamine-bonded silica lie in the ease of preparation of the stationary phase and the low cost of the silica gel.

EXPERIMENTAL

Apparatus. — An SP 8000 Spectraphysics liquid chromatograph equipped with a Waters R-401 refractometer detector was used. Stainless steel columns (150 × 4.8 mm i.d.) were packed by using the classical slurry method. The water adsorption isotherm was determined by the "batch method", using a Prolabo agitator having alternative movements. The water contents of the liquid phase were measured by the Karl Fisher method, using an E-547 automatic titrimeter Metrohm.

Stationary phases. — Copper silicate gel was prepared using Whatman Partisil 5 (mean particle size, 7 μm); for the water adsorption isotherms, Merck LiChrosorb Si60 (mean particle size, 40–60 μm) was used.

Silica gel was agitated for 10 min with ammoniated copper sulphate solution {$[Cu(II)] = 0.1$M, $[NH_3] =$ M}. The copper silicate gel was collected (Whatman filter GF/F, porosity 0.5μm), washed with M ammonia and then with distilled water, and dried at 100° for 12 h. Columns were filled by using the slurry technique, and then conditioned by washing with a water–acetonitrile mixture (120 mL, 25:75, $[NH_3] = 0.5$M).

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