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Note

In situ modification of silica with amines and its use in separating sugars by high-performance liquid chromatography

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One of the most convenient methods of sugar analysis at the present time involves a high-performance liquid chromatographic (HPLC) separation with refractive index (RI) monitoring of the eluate. This procedure has advantages over earlier gas chromatographic methods in that it is more rapid, requires no derivatisation, and the interpretation of results is simpler because the anomeric forms of sugars are not resolved by HPLC as they are by gas–liquid chromatography.

Sugar separations are best achieved on silicas that have been chemically changed to display a basic surface chemistry. A chemically bonded material marketed in the form of a "carbohydrate column" by Waters Assoc. (Milford, Mass., U.S.A.), was first introduced in 1974 and has found much subsequent use[1,2]. Comparable separations were achieved using silica modified by reaction with 3-aminopropyltriethoxysilane[3] and the reaction conditions have subsequently been studied in some detail[4]. More recently it has been shown that equally effective chromatography of sugars can be attained by "*in situ*" coating of silica using a poly-functional amine in the eluent[5].

Sugar analysis is of importance in forensic science mainly in the so-called "drugs intelligence" field. Illicit preparations are often diluted with sugars and it can be of interest to have information about their nature and relative proportions in order to compare various seizures. In studying this topic we have found that chemically bonded amino-phase packing materials tend to become "poisoned" when tablet and powder extracts are injected, a phenomenon characterised by a progressive deterioration in resolution. In our experience use of the *in situ* coating technique described by Aitzetmüller[5] produces columns displaying greater long-term stability and, in the work reported here, we have studied the separating capability of silica modified with a variety of amines.

EXPERIMENTAL

The stainless-steel columns used in this study were 12.5 cm × 0.49 cm I.D. × 1/4 in. O.D. terminated with zero-dead-volume reducing unions (1/4–1/16 in.) and were packed with an irregular silica of *ca.* 5 μm diameter, average pore diameter 13 nm, and surface area 320 m^2/g. Chemically bonded stationary phases prepared from the same base silica by reaction with 3-aminopropyltriethoxysilane, or 3-(2-aminoethylamino)-

propyltrimethoxysilane, were also studied (the organic loadings of the two packing materials were 7.5% and 14%, respectively)[4]. The columns packed with silica were treated by pumping an eluent consisting of acetonitrile–water (75:25) containing 0.1% of the amine for 0.5 h at 3 ml/min. The eluent was then changed to contain 0.01% of the amine and was pumped at 2 ml/min until equilibrium conditions were attained (about 30 min). Samples were dissolved in water to give an approximately 5–10% solution and 5 μl aliquots were injected using a stop-flow technique. The eluate was monitored with an Model 750/13 RI detector (Applied Chromatography Systems, Luton, Great Britain).

The amines studied were primary, secondary and tertiary butylamine, an homologous series of *n*-alkylamines, a diamino *n*-alkyl series with terminal amino groups in the 1,2 (*i.e.* ethylenediamine, EDA), 1,3, 1,5, 1,6 and 1,8 positions, and a polyamine series, diethylenetriamine, (DETA), triethylenetetramine (TETA), tetraethylenepentamine (TEPA) and pentaethylenehexamine (PEHA).

Pure sugar standards were used for much of the study but illicit drug preparations were examined by mixing with water, centrifuging and injecting a suitable aliquot of the supernatant.

RESULTS AND DISCUSSION

The findings of this study confirm the work of Aitzetmüller[5] in that they show that silica modified *in situ* with eluents containing polyfunctional amines can provide useful separations of sugars. The efficiency of the *in situ* coated columns and those

TABLE I

THE RETENTION DATA FOR SUGARS ON CHEMICALLY BONDED AND *IN SITU* MODIFIED PACKING MATERIALS

Chromatographic conditions: acetonitrile–water (75:25) + 0.01% amine at 2 ml/min, pressure drop *ca.* 900 p.s.i., column 12.5 cm × 0.49 cm I.D. k' = phase capacity ratio, k_o = 1.5 min.

Packing material or the amine used in the eluent	*k' Values for various sugars*					
	Fructose	*Mannitol*	*Glucose*	*Sucrose*	*Maltose*	*Lactose*
3-Aminopropyl*	1.7	2.2	2.3	3.7	5.0	5.5
3-(2-Aminoethylamino)propyl*	1.5	1.9	2.2	3.6	4.8	4.8
n-Butylamine	0.8	1.2	0.8	0.8	1.6	2.0
Dibutylamine	0.5	0.9	0.5	1.2	1.3	1.4
Tributylamine	0.6	1.0	0.6	1.2	1.3	1.4
n-Octylamine	0.8	1.2	0.8	1.3	1.5	1.9
n-Dodecylamine	0.8	1.2	0.8	1.3	1.6	2.0
1,3-Diamino	1.5	1.9	1.9	2.9	3.8	5.0
1,5-Diamino	1.5	1.9	1.9	2.9	3.8	4.6
1,6-Diamino	1.2	1.5	1.6	2.2	3.0	3.6
1,8-Diamino	0.9	1.2	0.9	1.4	1.8	2.1
EDA	1.3	1.7	1.5	2.3	3.0	3.7
DETA	1.4	1.9	1.9	2.6	3.5	4.2
TETA	1.5	1.9	1.9	2.9	3.8	4.6
TEPA	1.6	2.0	2.0	3.2	4.3	5.0
PEHA	1.3	1.7	1.7	2.7	3.5	4.1

* Bonded phase materials (no amine used in the eluent).

produced with a chemically bonded amino phase were rather similar (*e.g.* number of theoretical plates 1400–2000, *i.e.* 0.09–0.06 mm plate heights). Although not high by HPLC standards, this type of column performance is typical of that previously reported for sugars. At flow-rates higher than 2 ml/min the detector response decreased, hence this flow-rate was used throughout in order to provide a fast analysis with an acceptable response. The retention data for a variety of sugars are shown in Table I and characteristic chromatograms obtained with the series of diamines and polyamines are shown in Figs. 1 and 2. Fig. 3 compares a sugar separation on an aminopropyl bonded phase with that obtainable on silica before and after *in situ* modification with TEPA.

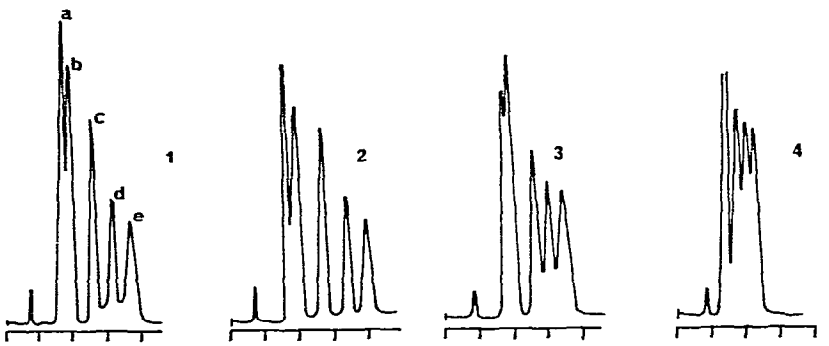

Fig. 1. The separation of (a) fructose, (b) glucose, (c) sucrose, (d) maltose, and (e) lactose on silica modified with diamines. Column: 12.5 cm × 0.49 cm I.D. Solvent: acetonitrile–water (75:25) + 0.01% amine at 2 ml/min. Amines studied: (1) 1,3-diaminopropane, (2) 1,5-diaminopentane, (3) 1,6-diaminohexane, (4) 1,8-diaminooctane. Detector: RI, time intervals 2 min.

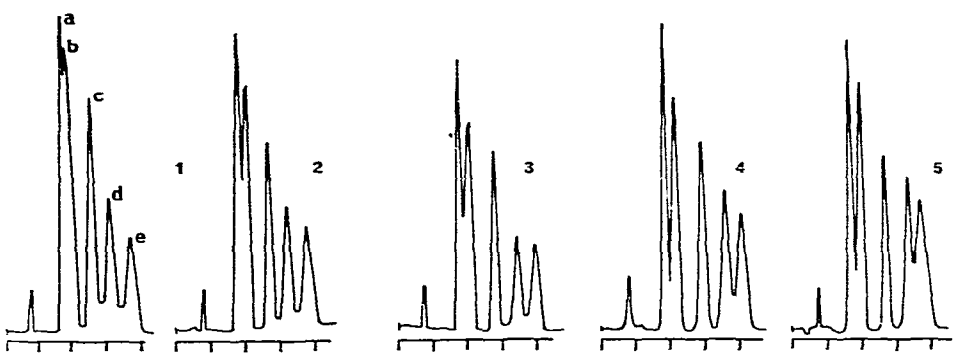

Fig. 2. The separation of sugars on silica modified with polyamines. The sugars and column conditions as indicated in Fig. 1. Amines studied: (1) EDA, (2) DETA, (3) TETA, (4) TEPA, (5) PEHA.

Coating silica with amines using the described procedure leads to greater retention of sugars than is the case when using the uncoated silica. However, the retention of sugars on such columns is somewhat lower than that obtained with chemically bonded amino phases. In general, the retention decreases in the sequence 3-aminopropyl bonded phase > 3-(2-aminoethylamino)propyl bonded phase > polyamines > diamines > amines > silica.

Primary aliphatic amines are of relatively little value for separating sugars

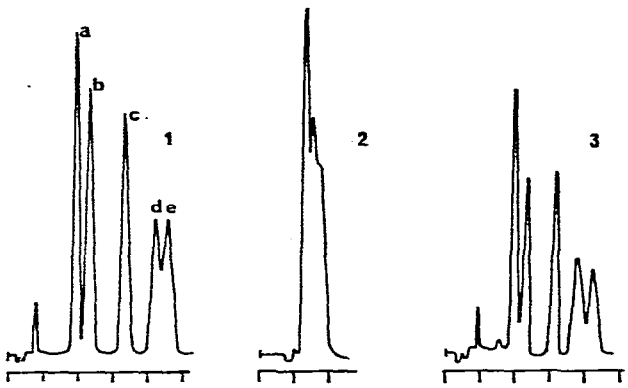

Fig. 3. The separation of sugars on (1) 3-aminopropyl bonded phase and (2) silica using an acetonitrile–water (75:25) eluent. Separation (3) was obtained on silica with 0.01% TEPA added to the eluent. Other conditions as in Fig. 1.

by this method. They do, however, partially resolve mannitol from glucose, and this is a mixture we have encountered in some illicit preparations. In the case of the diamines, those with chain-lengths up to C_5 were able to modify the silica so that it could be used to resolve the disaccharides, sucrose, maltose and lactose, but it was not possible to obtain baseline separations of the monosaccharides, glucose and fructose, on 12.5 cm columns with the eluent used. At chain-lengths greater than C_5, the overall retention of the sugars decreased. In the case of the polyamines the resolution obtained with the monosaccharides was somewhat improved, whereas that between maltose and lactose deteriorated. For the five common sugars, TEPA seems to provide an optimum separation. It is possible to obtain baseline resolution of all five common sugars on a 12.5 cm column by using a TEPA-containing eluent of lower water content, but this can only be attained at the expense of increased analysis time.

The most useful feature of the *in situ* coating method is that the columns prepared by this process are far more tolerant of non-sugar coextractives than are

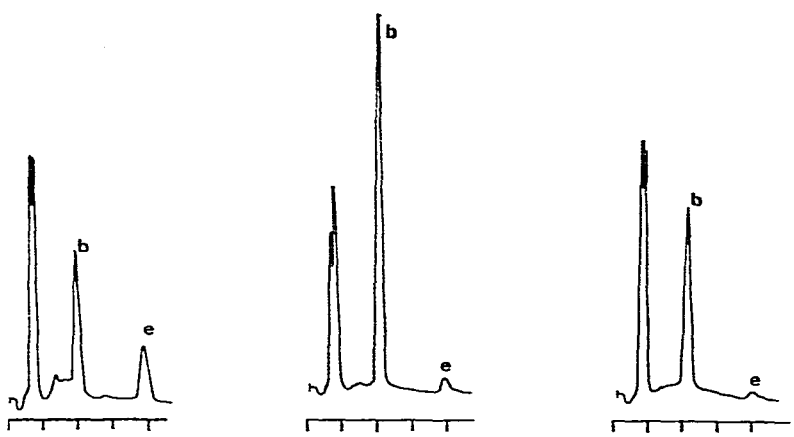

Fig. 4. Typical chromatograms of illicit powder extracts separated on silica using acetonitrile–water (75:25) containing 0.01% TEPA. All other conditions as in Fig. 1.

bonded-phase amino packings. This can be attributed to the fact that the adsorbed amine layer bound to the silica is continually being regenerated by the amine in the eluent. This type of dynamic equilibrium provides an inexpensive basis for preparing columns for sugar analysis having long-term stability; typical analyses are shown in Fig. 4. It has also been found that bonded amino-phase packing materials could be given an extended useful life by using an eluent containing TEPA.

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