Notes

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Liquid–solid chromatography with open glass capillary columns Separation of 1-dimethylaminonaphthalene-5-sulphonyl amino acids

Open capillary columns are very commonly used for gas–liquid chromatographic separations of complex mixtures[1]. Alkali-treated glass capillary columns have proved particularly useful in fractionation of gaseous isotopic mixtures[2–4]; their extremely high resolving ability is a consequence of a very effective partition between the gas phase and solid microlayer of silica gel deposited on the inner glass surface[2].

The success of these columns in gas–solid chromatography and the wide range of applications of silica gel in liquid–solid chromatography prompted us to test alkali-treated glass capillary columns for submicroscale separations not feasible by gas chromatographic methods.

The present paper represents the first contribution to this problem, namely the fractionation of DNS*-amino acids. These compounds have been chosen because of the strong yellow fluorescence, which easily allows one to detect very minute amounts throughout the operation of the column and, after elution, on thin-layer chromatograms. Furthermore a series of solvent systems suitable for their fractionation on silica gel plates has been described[5,6].

Experimental

Preparation of capillary columns. Capillary columns of various lengths and diameters were prepared from Murano soft-glass tubes (2.0 mm I.D. and 6.0 mm O.D.) using the glass-tube drawing apparatus of DESTY *et al.*[7]. The columns filled with 2.5 *N* NaOH were kept at 100° for different periods of time (from 2 to 8 h), then washed with water until neutral, rinsed with acetone and dried in a stream of nitrogen.

Injector. With a few modifications to be discussed later, the injection apparatus (Fig. 1) is essentially the same as used in gas chromatography.

Separation of DNS-amino acids. The columns were equilibrated with benzene–pyridine–acetic acid (80:20:2), one of the solvent systems suggested by MORSE AND HORECKER[5] for thin-layer chromatography of DNS-amino acids. After equilibration the needle valve was set to give a 1:30 ratio of flow rate in the column to flow rate in the valve. The flow rate of the column was kept constant at 0.5 ml/h by applying a pressure from 20 to 100 Torr.

By means of an Hamilton microsyringe, 4 μl of a DNS-amino acid mixture containing 6 μmoles/ml of each derivative, dissolved in benzene–pyridine–acetic acid (80:20:2), were then injected into the column. Owing to the low ratio of flow rate in the column to flow rate in the needle valve, the actual amount of each compound entering the column was less than 1 nmole. Each falling drop emerging from the column was examined by thin-layer chromatography on pre-coated silica gel plates (Merck) developed with the above-mentioned benzene–pyridine–acetic acid solvent system.

* Abbreviation: DNS = 1-dimethylaminonaphthalene-5-sulphonyl.

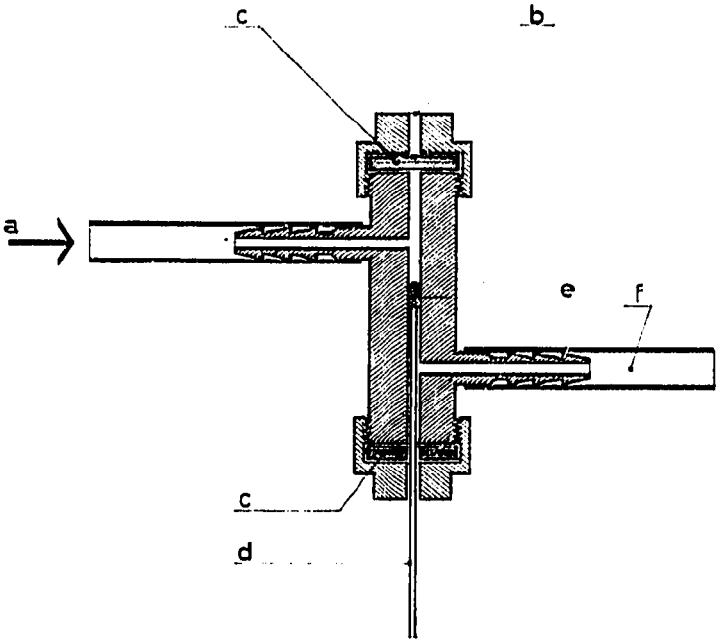

Fig. 1. Injector: a = solvent inlet, b = sample inlet, c = silicone rubber diaphragm, d = capillary column, e = platinum sponge, f = connection to needle valve.

R_F values were then plotted *vs.* time of appearance in the column eluate; in this way a qualitative elution pattern was obtained where each segment corresponds to the time interval during which one of the components emerges from the column. Such a "primitive" method of detection was quite satisfactory for this preliminary investigation, but the authors are aware that further developments will require the use of a highly sensitive detector capable of yielding a continuous recording of the solute concentration in the effluent liquid.

Results

Preliminary experiments showed that the method of introduction of the sample, as in gas–liquid chromatography, had a critical influence on the efficiency of the chromatographic procedure described in this paper. In fact, the use of an injection system without a needle valve, similar to that described by NYSTROM[8], gave poor results. By following the fluorescence of the samples traveling through the column, a remarkable broadening of the zones was apparent, even when the solutes were contained in a very small volume (0.2 μl).

With the use of a needle valve, which increases the flow rate between the injection site and the top of the column, sharp zones were obtained. Experiments run with different ratios of flow rate in the column to flow rate in the valve showed that a value of 1:30 was the most appropriate when the rate of flow of the column was 0.5 ml/h. The reproducibility of the chromatographic separations was improved when a small platinum sponge was inserted on top of the column (Fig. 1); this helped in getting a rapid mixing between sample and elution system.

The influence on the column efficiency of the size of the internal diameter was investigated by running a mixture of DNS-phenylalanine and DNS-glycine through columns treated 2 h with alkali and having identical lengths (10 m) but variable internal

diameters (0.23, 0.26 and 0.30 mm). In each case the flow rate was kept constant at 0.5 ml/h. The best results were obtained when the I.D. was 0.26 mm; in fact the time interval between the disappearance from the eluate of the fluorescence due to DNS-phenylalanine and the appearance of that due to DNS-glycine was 9, 14 and 4 min for columns with an I.D. of 0.23, 0.26 and 0.30 mm, respectively. In each case the zone width was unchanged.

Similarly, the effect of time of heating with alkali on the column efficiency was investigated. On 2-m-long columns, the two above-mentioned DNS-amino acids were not resolved when the attack with alkali had lasted only 2 h; with a 5-h treatment their separation corresponded to 5 min, a value which remained unchanged when the period of heating with NaOH was further extended.

Fig. 2 depicts the elution pattern of nine DNS-amino acids under the best

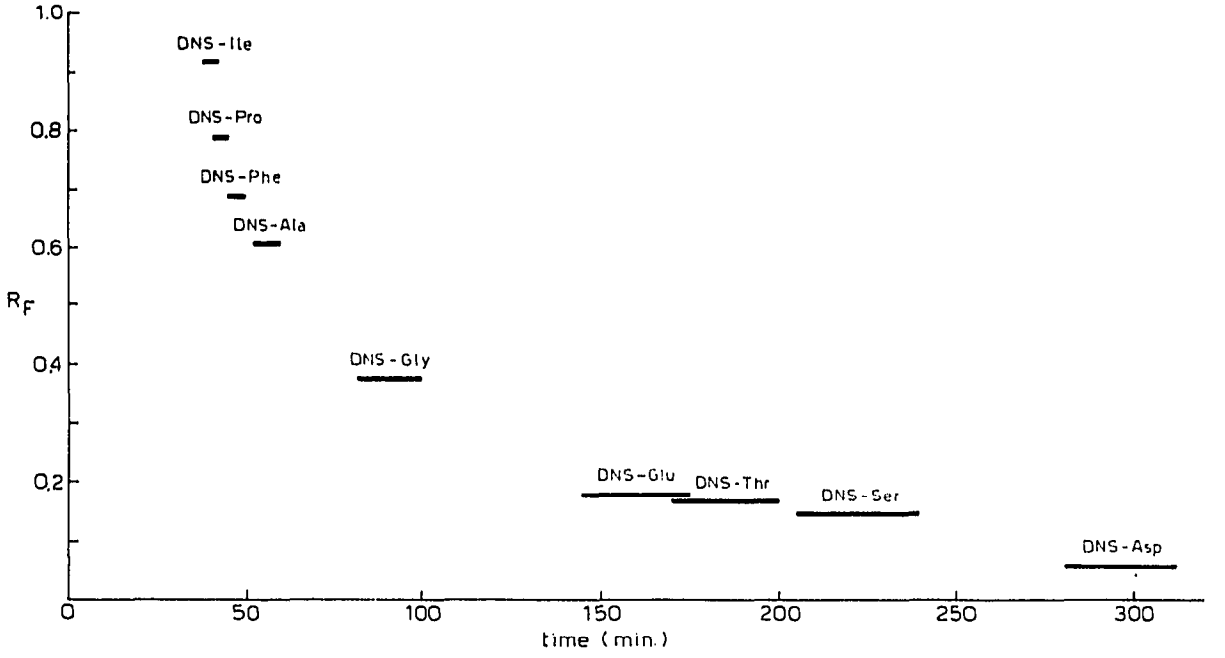

Fig. 2. Elution pattern of DNS-amino acids from the capillary column (see *Experimental*).

conditions worked out through the experiments reported above, namely: flow rate, 0.5 ml/h; ratio of flow rate in the column to flow rate in the needle valve, 1:30; I.D., 0.26 mm; length, 5 m; time of heating with alkali, 5 h. The separation of the nine derivatives is nearly complete, and, as compared to thin-layer chromatography in the same solvent system, is particularly good for those having low R_F values.

Discussion

The experiments reported in the present paper show that alkali-treated glass capillary columns can be used successfully for liquid–solid chromatographic separations. As compared to packed columns, these open columns work at exceedingly low pressure; therefore their length can be enormously increased, with the consequence that the number of theoretical plates, *i.e.* the resolution, attains very high values. This peculiar property is common to the capillary columns so widely used in gas–chromatographic analyses.

DNS-amino acids have proved particularly appropriate for this preliminary investigation, aimed to ascertain the potentiality of capillary columns in liquid–solid chromatographic separations. In fact the strong fluorescence of these compounds permits one to follow the formation of the zones and their behaviour throughout the whole operation and to detect in the eluate, without resorting to special equipment, the minute amounts of solute compatible with the capacity of capillary columns. Hence, a simple injection system, capable of yielding very sharp zones, has been developed and the main parameters (length and internal diameter of the column, extent of treatment with alkali) influencing the efficiency of the column have been fixed.

The reproducibility of the results has been excellent; in particular, with the solvent system used in the present investigation (benzene–pyridine–acetic acid), the same column can be used for subsequent runs of DNS-amino acid mixtures without impairing its efficiency. The positive results obtained in this first approach to the utilisation of capillary columns for liquid–solid separations are very encouraging. In fact, the high resolution, the speed of the operation and the requirement of minute amounts of sample make this technique very attractive. Furthermore, the availability of capillary columns coated with different types of adsorbent[9–12], as well as the obvious possibility of continuously recording the effluent using a sensitive detector, suggests potential applications for a very large number of analytical problems.

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1 I. HALÀSZ, K. HARTMANN AND E. HEINE, in A. GOLDUP (Editor), *Gas Chromatography 1964*, Institute of Petroleum, London, 1965, p. 38.
2 M. MOHNKE AND W. SAFFERT, in M. VAN SWAAY (Editor), *Gas Chromatography 1962*, Butterworths, London, 1962, p. 216.
3 F. A. BRUNER AND G. P. CARTONI, *Anal. Chem.*, 36 (1964) 1522.
4 W. BOCOLA, F. A. BRUNER AND G. P. CARTONI, *Nature*, 209 (1966) 200.
5 D. MORSE AND B. L. HORECKER, *Anal. Biochem.*, 14 (1966) 429.
6 Z. DEYL AND J. ROSMUS, *J. Chromatog.*, 20 (1965) 514.
7 D. H. DESTY, T. M. HARESNAPE AND B. H. F. WHYMAN, *Anal. Chem.*, 32 (1960) 302.
8 E. NYSTRÖM AND J. SJÖVALL, *J. Chromatog.*, 24 (1966) 212.
9 I. HALÀSZ AND C. HORVÀTH, *Anal. Chem.*, 35 (1963) 499.
10 I. HALÀSZ AND C. HORVÀTH, *Anal. Chem.*, 36 (1964) 2226.
11 I. HALÀSZ AND C. HORVÀTH, *Ger. Pat.*, 1, 183, 716; *C.A.*, 62 (1965) 9800 h.
12 G. C. GORETTI, A. LIBERTI AND G. NOTA, *J. Chromatog.*, 34 (1968) 96.

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