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Note

Gas–liquid chromatography of amino acids with Supelcoport as solid support

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It is well known[1] that there are major differences between gas chromatographic supports based on diatomaceous earth. Apart from the origin of this raw material (*viz.*, fresh water or marine deposits), conditions used in the flux calcining process, which *inter alia* reduces the surface area, govern important properties (*e.g.* density) of the support type. Subsequent procedures to modify the surface characteristics of support particles (*e.g.* acid and/or base washing and/or silanising) also contribute to differences in performance which characterise materials currently commercially available.

The so-called "white supports" which include, for example, Chromosorb W, Supelcoport and Gas-Chrom Q, have long been known to be extremely fragile and susceptible to fragmentation during transportation. Considerable care must be exercised in their handling to avoid "fine" formation and exposure of "active sites" both of which lead to suboptimal chromatographic performance, more especially encountered when dealing with separation of sensitive compounds.

The N-acyl alkyl ester derivatives of amino acids are an example of such compounds and for some years, on account of its constant good quality, we have used Supelcoport as support in our quantitative analysis of N-heptafluorobutyryl isobutyl ester (HBB) derivatives of amino acids. In the light of foregoing observations regarding fragility, newly purchased consignments of Supelcoport are routinely placed on a fluid bed to remove "fines" and resilanised to ensure deactivation of any "active sites". Only then is the support coated with liquid phase (*viz.*, OV-101) using previously described techniques[2].

During 1984 we started to observe selective peak broadening, in the case of the arginine derivative, which develops after as few as 50 analyses of standards. Previous experience had shown that a column could be expected to last for up to 700 analyses before showing signs of a more general deterioration. The arginine broadening is also accompanied in its later stages by diminishing peak height, the overall shape (Fig. 1) being indicative of adsorption on column packing.

Bulk density of support materials is an important criterion used in their quality control, large variations rendering such products unacceptable. Use of Supelcoport is claimed to minimise batch-to-batch variations in bulk density and particle size. Along with arginine peak deterioration, we observed that the bulk density of different

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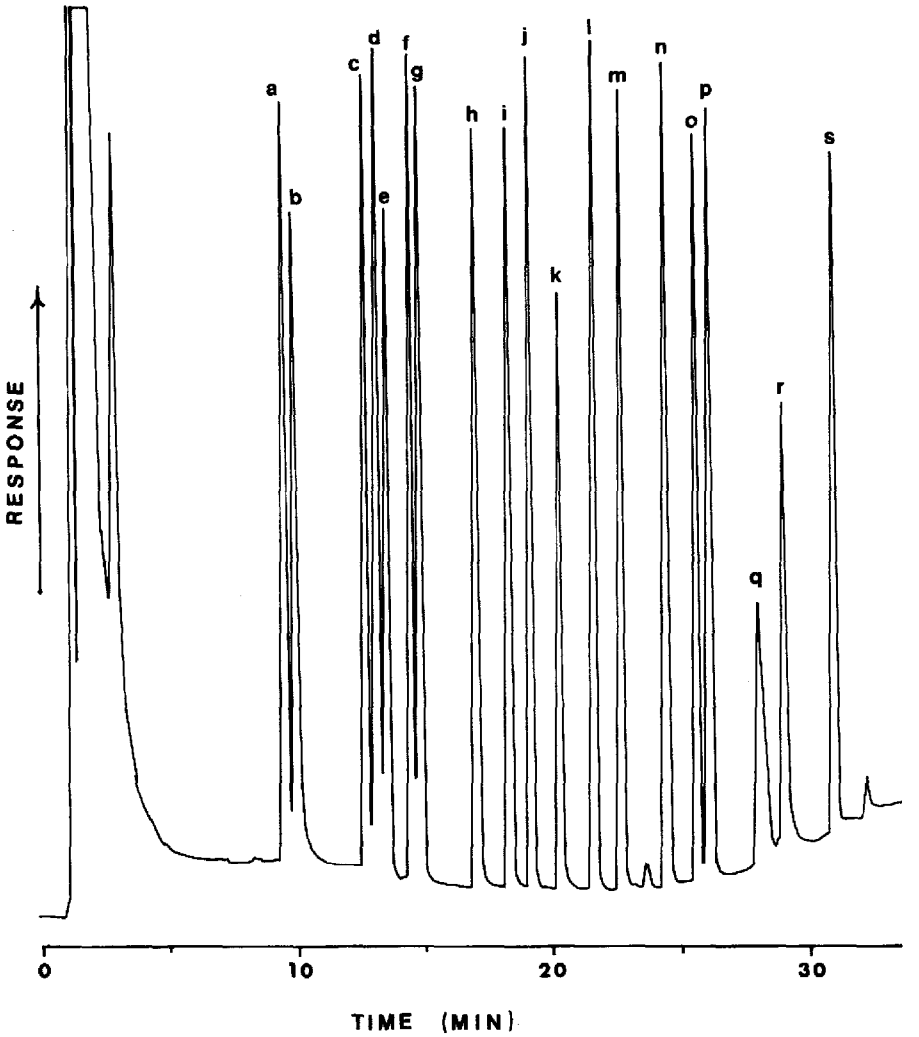

Fig. 1. Chromatogram of HBB derivatives of amino acids illustrating broadening and shortening of the arginine peak. Obtained using a column packed with Supelcoport (100–120 mesh) coated in this laboratory with OV-101 (3.5% loading). Peak identification: a = alanine; b = glycine; c = valine; d = threonine; e = serine; f = leucine; g = isoleucine; h = proline; i = pipecolic acid (internal standard); j = hydroxyproline; k = methionine; l = aspartic acid; m = phenylalanine; n = glutamic acid; o = lysine; p = tyrosine; q = arginine; r = histidine; s = tryptophan.

production batches of Supelcoport varied by as much as 30% (*viz.*, 0.20 to 0.26 g/ml). Whilst this *per se* does not account for selective behaviour of the type encountered, such variation does throw considerable doubt on the integrity of the packing material in general.

We have been advised[3] that Supelcoport is now no longer available as an uncoated material. On account of various problems which have occured during preparation of this support, it appears that in future only pre-coated and pre-tested ma-

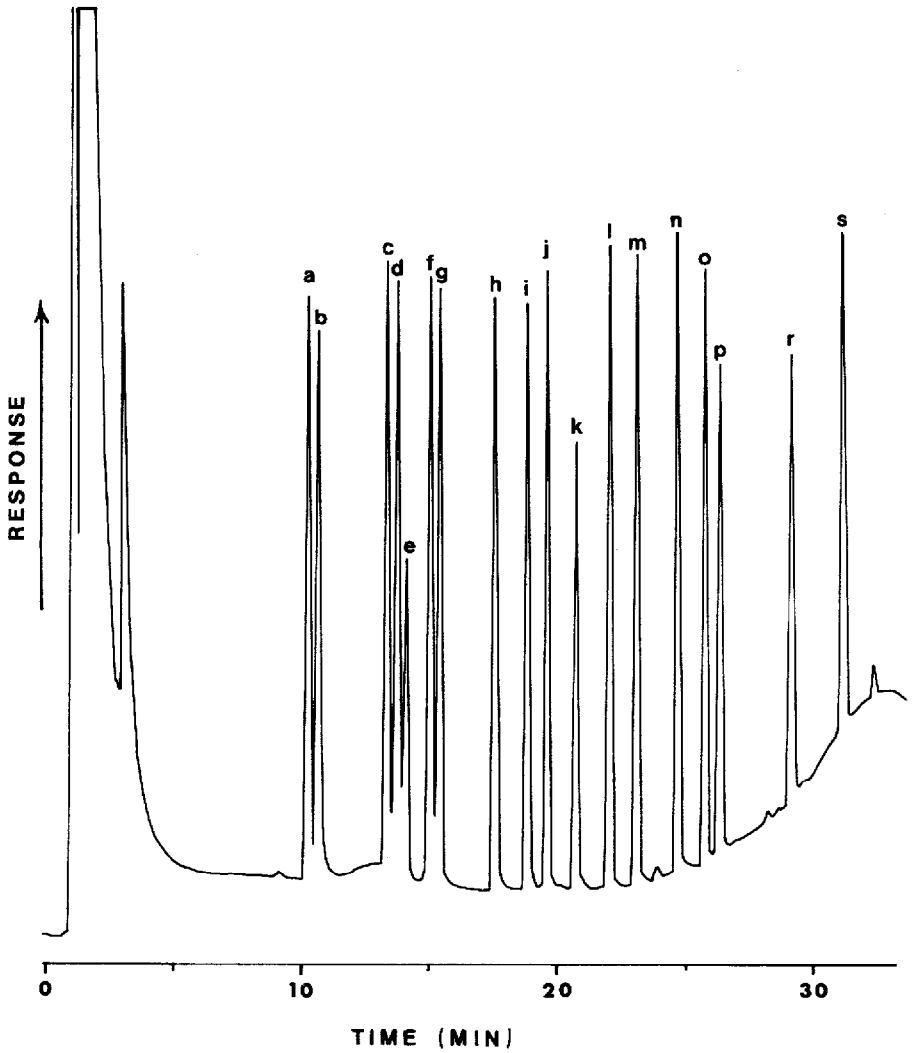

Fig. 2. Chromatogram of HBB derivatives of amino acids obtained using a column packed with recently received commercially pre-coated (3.5% OV-101) Supelcoport. Peak identification as in Fig. 1.

terials will be available. In view of their fragility, the value of such loose packings, following transportation over long distances, may be doubtful.

A recently received sample of Supelcoport pre-coated with OV-101 yielded a chromatogram of HBB amino acid derivatives (Fig. 2) which differs in three significant features from previous analyses (Fig. 3). Firstly, there is no arginine peak; secondly, response to serine is greatly diminished and thirdly, all retention times are longer by approximately 1.75 min. The first two observations lead to the conclusion that the most recent batches of Supelcoport display considerably more activity than that previously experienced and that this is responsible for adsorption of *inter alia*

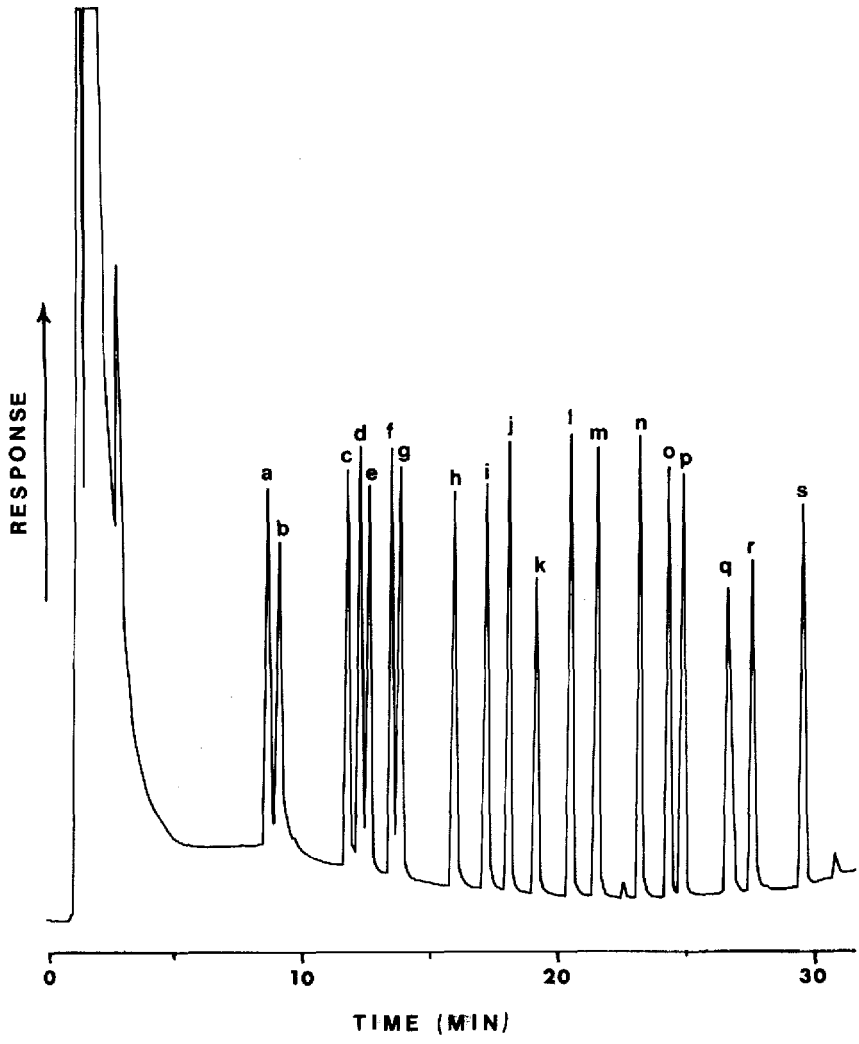

Fig. 3. Chromatogram of HBB derivatives of amino acids obtained using a column packed with Supelcoport (100–120 mesh), from an early batch, coated in this laboratory with OV-101 (3.5% loading). Peak identification as in Fig. 1.

the arginine derivative, a phenomenon which was not apparent in much earlier batches. The third observation is due to an increased quantity of liquid phase in the column as a consequence of an increase in support density.

In view of the unsatisfactory nature of currently available Supelcoport, we evaluated an alternative support material. Chromosorb W-HP may offer a solution to this difficulty as judged by a typical chromatogram (Fig. 4) and data derived from quantitative analyses (Table I) performed on a column containing packing based on this support. Comparison with similar data (Table II), obtained from a column prepared from an earlier batch of Supelcoport, suggests that Chromosorb W-HP is an acceptable alternative allowing quantitative determination of arginine.

TABLE I

QUANTITATIVE PERFORMANCE OF A COLUMN PACKED WITH SUPELCOPORT (100–120 MESH) COATED WITH 3.5% OV-101

Amino acid	*Mean response**	*S.D.*	*R.S.D.(%)*
Alanine	1.049	0.004	0.38
Glycine	1.039	0.024	2.31
Valine	1.036	0.001	0.11
Threonine	0.953	0.003	0.31
Serine	0.990	0.014	1.39
Leucine	1.030	0.001	0.10
Isoleucine	0.994	0.002	0.25
Proline	1.009	0.002	0.22
Hydroxyproline	0.932	0.007	0.77
Methionine	1.531	0.015	0.98
Aspartic acid	0.932	0.005	0.49
Phenylalanine	0.949	0.002	0.23
Glutamic acid	0.911	0.003	0.28
Lysine	1.010	0.007	0.67
Tyrosine	0.999	0.008	0.79
Arginine	1.088	0.044	4.01
Histidine	1.361	0.042	3.11
Tryptophan	1.836	0.008	0.46

* Mean of six injections of the same sample of amino acid standard derivatives.

TABLE II

QUANTITATIVE PERFORMANCE OF A COLUMN PACKED WITH CHROMOSORB W-HP (100–120 MESH) COATED WITH 3.5% OV-101

Amino acid	*Mean response**	*S.D.*	*R.S.D. (%)*
Alanine	1.090	0.004	0.34
Glycine	1.095	0.024	2.19
Valine	1.035	0.008	0.75
Threonine	0.902	0.006	0.68
Serine	1.213	0.036	2.99
Leucine	0.993	0.003	0.34
Isoleucine	0.948	0.002	0.22
Proline	1.003	0.005	0.48
Hydroxyproline	1.010	0.009	0.87
Methionine	1.615	0.035	2.16
Aspartic acid	0.982	0.007	0.66
Phenylalanine	0.937	0.002	0.24
Glutamic acid	0.877	0.003	0.35
Lysine	0.962	0.004	0.45
Tyrosine	0.930	0.004	0.47
Arginine	1.189	0.026	2.21
Histidine	1.563	0.055	3.55
Tryptophan	1.447	0.007	0.48

* Mean of six injections of the same sample of amino acid standard derivatives.

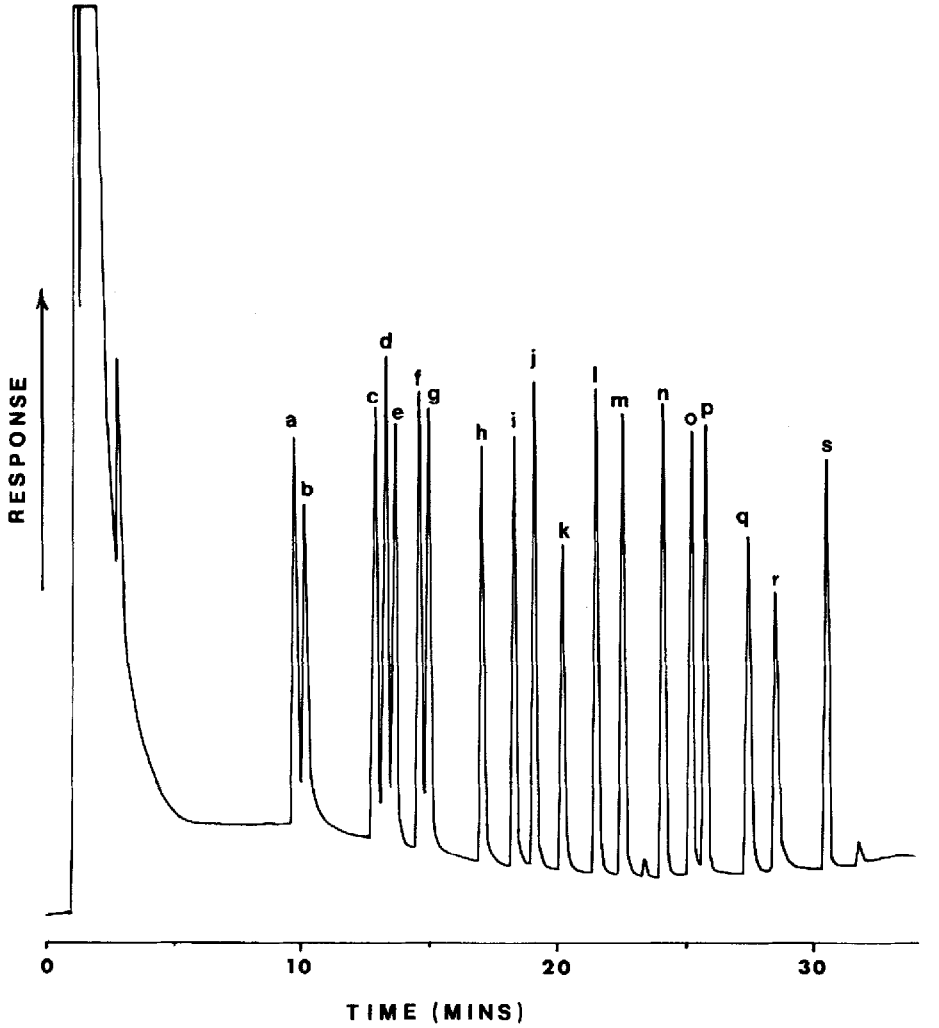

Fig. 4. Chromatogram of HBB derivatives of amino acids obtained using a column packed with currently available Chromosorb WHP (100–120 mesh) coated in this laboratory with OV-101 (3.5% loading). Peak identification as in Fig. 1.

ACKNOWLEDGEMENTS

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3 Supelco, Bellefonte, PA, private communication.