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Note

Retention behaviour of phenylthiohydantoin amino acids in micro high-performance liquid chromatography with octadecyl bonded glasses and silicas

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Reversed-phase high-performance liquid chromatography (HPLC) is one of the most frequently used liquid chromatography modes. In this technique, chemically bonded stationary phases are widely used, and the retention of solutes on, for example, *n*-octadecyl-silica (C_{18}) seems to be controlled by some factors related to the size and shape of the solutes. In previous papers[1–5], we have suggested that the important parameters of silica with respect to the number of accessible alkylamino or phenyl groups per 100 Å^2 are the pore diameter and the specific surface area. In investigations of bioavailability, as well as in pharmacokinetic and forensic science studies, several types of gel are used. Therefore, we have now considered how the chromatographic properties of octadecyl-modified HPLC glass gels compare with those of HPLC silica gels. Octadecyl-modified gels are important HPLC or micro-HPLC column gels as well as phenyl-modified gels[6–13], but there have been few reports of HPLC analyses on octadecyl-bonded glass columns in physical and chemical research[14–17]. We have also studied the preparation of four types of octadecyl-modified glasses or silicas, and evaluated their performance in the HPLC of twenty typical phenylthiohydantoin (PTH) amino acids.

EXPERIMENTAL

Reagents

Porous glass and porous silica were prepared in our laboratories (Table I). Octadecyldimethylchlorosilane (ODS) and trimethylchlorosilane (TMS) were obtained from Petrach Systems (PA, U.S.A.). Acetonitrile (HPLC grade) and twenty PTH amino acids were obtained from Wako (Osaka, Japan). The other reagents and organic solvents were of analytical grade.

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TABLE I

CHARACTERISTICS OF ORIGINAL GLASS AND SILICA

*Sample**	*Mean particle size (μm)*	*Mean pore diameter (Å)*	*Specific surface area (m^2/g)*	*Pore volume (ml/g)*
Glass 1G	8.9	150	154	0.85
Silica 1S	8.0	164	197	1.20

* The designations are for convenience and are not commercial names.

TABLE II

CHARACTERISTICS OF TREATED GLASSES AND SILICAS

Treated gel	*Specific surface area (m^2/g)*	*Carbon content (%)*	*Average pore diameter (Å)*	*Pore volume (ml/g)*
1G-ODS	103	12.5	116	0.53
1G-ODS-TMS	85	12.9	113	0.40
1S-ODS	146	11.0	130	0.81
1S-ODS-TMS	143	11.9	125	0.70

TABLE III

RETENTION DATA OF PTH AMINO ACIDS ON OCTADECYL-BONDED GLASSES AND SILICAS

Conditions as in Fig. 1.

PTH amino acids	*Capacity factor, k'*			
	1G-ODS	*1G-ODS-TMS*	*1S-ODS*	*1S-ODS-TMS*
Asp	0.17	0.23	0.23	0.20
Glu	0.20	0.30	0.26	0.28
Asn	0.41	0.46	0.51	0.49
Gln	0.52	0.55	0.66	0.58
Ser	0.47	0.51	0.56	0.61
His	0.40	0.45	0.94	0.41
Arg	0.49	0.49	1.31	0.41
Gly	0.63	0.77	0.94	1.05
Ala	1.15	1.14	1.42	1.60
Tyr	1.38	1.42	1.77	2.31
Thr	1.91	1.77	2.35	3.07
Val	2.73	3.36	3.02	4.15
Nor	3.28	3.61	3.45	4.75
Pro	2.67	2.81	3.40	4.18
Met	2.73	2.91	2.36	4.24
Ile	4.88	4.72	5.03	7.41
Leu	5.53	5.87	5.63	8.45
Phe	5.12	6.24	5.92	7.94
Trp	4.67	4.22	5.27	7.89
Lys	6.06	6.27	6.95	10.50

TABLE IV

SEPARATION EFFECT OF TMS ENDCAPPING ON GLASSES AND SILICAS

Conditions as in Fig. 1.

Treated gel	*Separation factor,* $\alpha = k'_A/k'_B$		
	PTH-Ser vs. PTH-His	*PTH-Gly vs. PTH-Arg*	*PTH-Met vs. PTH-Pro*
1G-ODS	1.18	1.29	1.02
1G-ODS-TMS	1.13	1.57	1.04
1S-ODS	0.60	0.72	0.69
1S-ODS-TMS	1.49	2.56	1.01

Apparatus

The HPLC measurements were carried out using a microfeeder MF-2 (Azuma Electric, Tokyo, Japan) equipped with a Model 8000 UV detector (Toyo soda, Tokyo, Japan).

Stationary phase and elemental analysis

As described previously[16,17], 7 g of dried glass 1G or silica 1S were added to 70 ml of a 3.4% solution of ODS in dry toluene containing 3 ml of triethylamine. The glass or the silica suspension was refluxed for 5 h, filtered with a glass filter (1 μm), washed several times with toluene, chloroform, methanol and acetone and then dried *in vacuo* at 70°C for 2 days. The final products are listed in Table II as 1G-ODS and 1S-ODS, respectively. A 3.5-g amount of 1G-ODS or 1S-ODS was added to 35 ml of a 3.4% toluene solution of TMS for endcapping, and the same procedure was carried out as above, producing 1G-ODS-TMS and 1S-ODS-TMS. The characteristics of these materials are also given in Table II.

The carbon contents of the treated glasses or silicas were determined by elemental analysis using an MT-3 CHN elemental analyser (Yanagimoto, Kyoto, Japan). The specific surface areas, mean pore diameters and pore volumes of the column glasses and column silicas were determined with an MOD-220 porosimeter (Carlo Erba, Milan, Italy) and an SA-1000 surface-area pore-volume analyser (Shibata, Tokyo, Japan). All the data are listed in Table II.

TABLE V

TMS ENDCAPPING EFFECT ON THE SEPARATION OF PTH-Gln AND PTH-Ser

Conditions as in Fig. 1.

Treated gel	$\alpha = k'_{PTH\text{-}Gln}/k'_{PTH\text{-}Ser}$
1G-ODS	1.11
1G-ODS-TMS	1.08
1S-ODS	1.18
1S-ODS-TMS	0.95

TABLE VI

IMPROVEMENT OF SEPARATION ON GLASSES *VERSUS* SILICAS

Conditions as in Fig. 1.

Treated gel	*Separation factor,* $\alpha = k'_A/k'_B$	
	PTH-Phe vs. PTH-Trp	*PTH-Leu vs. PTH-Trp*
1G-ODS	1.10	1.18
1G-ODS-TMS	1.48	1.39
1S-ODS	1.12	1.07
1S-ODS-TMS	1.01	1.07

Column preparation

The columns were 500 mm × 0.32 mm I.D. fused-silica capillaries packed by the slurry technique.

RESULTS AND DISCUSSION

Table III shows the capacity factors, k', of twenty PTH amino acids on 1G-ODS, 1G-ODS-TMS, 1S-ODS and 1S-ODS-TMS. The PTH amino acids were sep-

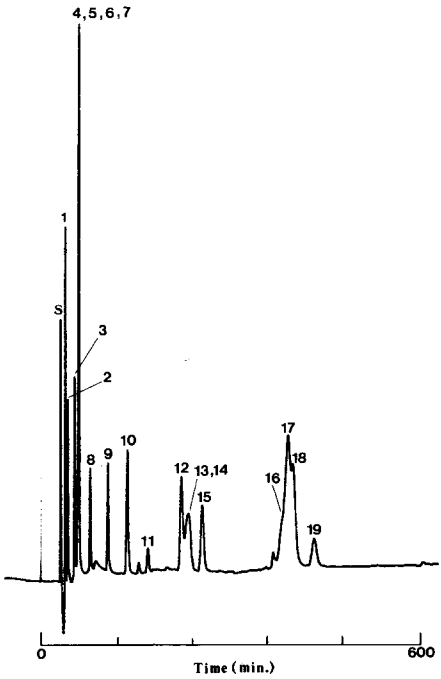

Fig. 1. Typical chromatogram obtained with typical PTH amino acids on 1S-ODS-TMS. Column: 500 mm × 0.32 mm I.D.; mobile phase: 30% acetonitrile in tetrahydrofuran–0.01 *M* sodium acetate buffer, pH 5.4 (2:98, v/v); flow-rate: 2 μl/min; UV detection: 254 nm. PTH amino acids: 1 = L-aspartic acid; 2 = L-glutamic acid; 3 = L-arginine hydrochloride; 4 = L-histidine hydrochloride; 5 = L-asparagine; 6 = L-glutamine; 7 = DL-serine; 8 = DL-glycine; 9 = DL-α-alanine; 10 = L-tyrosine; 11 = *Δ*-threonine; 12 = DL-valine; 13 = L-proline; 14 = DL-methionine; 15 = DL-norvaline; 16 = DL-isoleucine; 17 = DL-tryptophan; 18 = L-phenylalanine; 19 = L-leucine.

arated on the glasses as well as silicas studied, but with different degrees of resolution and elution orders.

By comparison of ODS-treated glasses and silicas, the separation factors, α, were measured under the same HPLC conditions. Table IV shows that the separation of PTH-Ser *vs.* PTH-His, PTH-Gly *vs.* PTH-Arg and PTH-Met *vs.* PTH-Pro, due to the influence of silanol groups, was worse on 1S-ODS than on 1S-ODS-TMS; on glass the effect of endcapping with TMS was less pronounced.

Table V shows that the resolution of PTH-Gln *vs.* PTH-Ser on 1G-ODS and 1S-ODS was improved compared with that on 1G-ODS-TMS and 1S-ODS-TMS.

On the other hand, from Table VI, it is seen that the resolution of PTH-Phe *vs.* PTH-Trp and of PTH-Trp *vs.* PTH-Leu on ODS-treated glass gels with TMS endcapping was improved compared with that on the ODS-treated silica series.

Fig. 1 shows a typical chromatogram obtained with typical PTH amino acids on 1S-ODS-TMS.

The data in Tables IV–VI reveal that silanols on glasses or silicas change the elution behaviour of some PTH amino acids. According to previous work[1–3,16], the numbers of accessible TMS surface groups per gram were calculated to be $0.0668 \cdot 10^{21}$ for glass and $0.1504 \cdot 10^{21}$ for silica. Thus the silanol groups on silicas are more reactive than those on glasses.

These results show that the ODS-modified glasses could be useful column materials for HPLC. Our investigations also indicate that, in the evaluation of column materials, the pore distribution of the support glass, the bulkiness of the ligand bonded to the glass and the molecular size of the solute must also be considered.

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