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Note

Thin-layer chromatography of amino acids and dipeptides on RP-2, RP-8 and RP-18 plates impregnated with dodecylbenzenesulphonic acid

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The usefulness of home-made layers of silanized silica gel (C_2) impregnated with anionic and cationic detergents in the separation of amino acids[1] and peptides[2,3] has already been demonstrated. Recently, a number of studies of commercially available plates pre-coated with silanized silica gel were carried out in order to determine the behaviour of such layers with different eluents[4–6] and their separation power for complex mixtures of organic compounds[4,6–9].

It was of interest therefore to study the chromatographic behaviour of amino acids and dipeptides on RP-2, RP-8 and RP-18 plates after their impregnation with dodecylbenzenesulphonic acid in order to determine the optimum conditions for the separation of these compounds and to compare the results with those obtained on home-made layers[1–3].

EXPERIMENTAL

RP-2, RP-8 and RP-18 plates (E. Merck, Darmstadt, G.F.R.) were dipped for 5 min in ethanol–water (96:4) containing dodecylbenzenesulphonic acid (HDBS) in the required percentage and then air-dried.

The migration distance was 6 cm unless otherwise stated. The measurements were carried out at 25°C.

The standard solutions of amino acids and peptides were obtained by dissolving the compounds in water–methanol (40:60) to give a concentration of 0.5 mg/ml. A 0.2-μl volume of each solution was deposited on the layers, with the exception of tyrosine for which 0.5 μl were used.

RESULTS AND DISCUSSION

Layers of RP-2, RP-8 and RP-18

In agreement with recent studies[4–6], the RP-2, RP-8 and RP-18 plates cannot be used with aqueous organic eluents containing more than 30% of water. Such eluents migrate so slowly that the time required for a run is prohibitively long. For example, elution with 1 *M* acetic acid in methanol–water (50:50) on RP-2 required a migration time of 100 min. Under such conditions all the compounds exhibited R_F

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values higher than 0.75. However, an increase of the methanol content results in a decrease both in the migration time and in the retention of the compounds. Elution with water–methanol (40:60) reduced the analysis time to 70 min, but did not involve remarkable changes in the retention of the different compounds, with the exception of histidine and glycylhistidine which are more strongly retained and give rise to elongated spots.

Layers of RP-2, RP-8 and RP-18 impregnated with dodecylbenzenesulphonic acid

The use of impregnated layers resulted in a sharp decrease in the migration time, as shown in Fig. 1 where the migration time (in minutes) on the RP-2 plates is plotted as a function of the HDBS content of the ethanolic solution. It should be noted that only for $\leqslant 1\%$ HDBS are no appreciable differences observed in the development time with respect to non-impregnated layers.

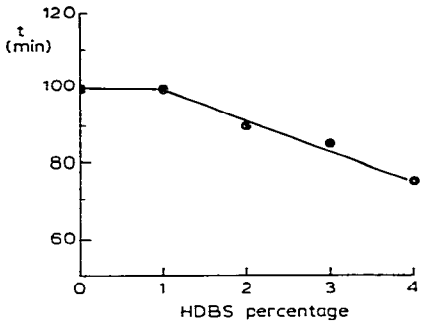

Fig. 1. Dependence of development time, t, over distances of 6 cm on RP-2 plates (alone or impregnated with increasing amounts of dodecylbenzenesulphonic acid), with 1 M acetic acid in methanol–water (50:50) as eluent.

The retention of the fourteen glycyl dipeptides and of the corresponding amino acids is marked on impregnated layers (*i.e.*, RP-2) even at low HDBS contents (*i.e.*, 1 %) and it is little affected by an increase of the detergent concentration. However, more compact spots are obtained by changing from 1 % to 4 % HDBS. For these reasons we used RP-2, RP-8 and RP-18 plates impregnated with 4 % HDBS.

The R_F values of the amino acids and dipeptides eluted with 1 M acetic acid in 50 % methanol and with 1 M acetic acid + 0.2 M hydrochloric acid in 50 % methanol are listed in Table I. It is interesting that on RP-2 and RP-8 plates the migration time is the same (about 75 min), but on RP-18 plates it decreases surprisingly to 45 min. The affinity of the stationary phase towards the different compounds remarkably increases on changing from RP-2 to RP-8 plates, but varies very little or remains constant on changing from RP-8 to RP-18. On the last layers the spots are much narrower and are therefore suitable for analytical applications.

The increase of the R_F values when hydrochloric acid is present in the eluent indicates the operation of an ion-exchange process in the retention mechanism similar to that observed on home-made layers[1–3]. As the hydrochloric acid concentration is increased (*i.e.*, to 0.5 M) the R_F values begin to level off.

The affinity sequence of the dipeptides and amino acids is the same on RP-2, RP-8, RP-18 and on home-made layers, notwithstanding the different composition of

TABLE I

R_F VALUES OF AMINO ACIDS AND DIPEPTIDES ON LAYERS OF RP-2, RP-8 AND RP-18 IMPREGNATED WITH 4% HDBS

Eluents: 1 = 1 *M* acetic acid in methanol–water (50:50); 2 = 1 *M* acetic acid + 0.2 *M* HCl in methanol–water (50:50).

Compound	*Eluent 1*			*Eluent 2*		
	RP-2 + 4% HDBS	*RP-8 + 4% HDBS*	*RP-18 + 4% HDBS*	*RP-2 + 4% HDBS*	*RP-8 + 4% HDBS*	*RP-18 + 4% HDBS*
Glu	0.40	0.26	0.25	0.72	0.66	0.66
Ser	0.38	0.24	0.21	0.72	0.65	0.63
Thr	0.37	0.21	0.19	0.70	0.61	0.61
Gly	0.35	0.20	0.16	0.68	0.60	0.57
Ala	0.32	0.18	0.14	0.65	0.56	0.53
Pro	0.28	0.13	0.13	0.58	0.49	0.44
Tyr	0.23	0.13	0.12	0.55	0.46	0.47
Val	0.20	0.08	0.06	0.51	0.38	0.36
Met	0.18	0.07	0.05	0.45	0.32	0.31
Ile	0.14	0.05	0.04	0.37	0.25	0.25
Leu	0.14	0.04	0.03	0.35	0.22	0.20
Phe	0.14	0.04	0.03	0.34	0.21	0.18
Trp	0.12	0.03	0.02	0.26	0.17	0.15
His	0.07	0.00	0.00	0.21	0.11	0.10
Gly-Glu	0.40	0.26	0.24	0.72	0.67	0.68
Gly-Ser	0.38	0.23	0.20	0.72	0.67	0.66
Gly-Thr	0.38	0.22	0.20	0.72	0.66	0.65
Gly-Gly	0.35	0.19	0.16	0.68	0.62	0.58
Gly-Ala	0.29	0.16	0.13	0.64	0.58	0.55
Gly-Pro	0.26	0.13	0.10	0.59	0.51	0.47
Gly-Tyr	0.25	0.13	0.11	0.56	0.51	0.53
Gly-Val	0.18	0.08	0.06	0.51	0.41	0.38
Gly-Met	0.17	0.07	0.05	0.47	0.36	0.34
Gly-Ile	0.12	0.04	0.04	0.36	0.25	0.25
Gly-Leu	0.12	0.03	0.03	0.34	0.22	0.23
Gly-Phe	0.12	0.03	0.03	0.32	0.21	0.21
Gly-Trp	0.12	0.03	0.02	0.31	0.21	0.20
Gly-His	0.04	0.00	0.00	0.25	0.13	0.12

the eluents[1–3]. The only exception was tyrosine and glycyltyrosine which on RP-18 plates were less strongly retained than proline and glycylproline, in contrast to the behaviour observed on the other layers. Elution with water–methanol mixtures without organic or mineral acids did not result in any improvements from an analytical standpoint with respect to the elution with acidic solutions, since the compounds are generally more strongly retained.

At high methanol percentages, *i.e.*, methanol–water (80:20) or with water–methanol–tetrahydrofuran (40:30:30) the R_F values increased, but the more polar amino acids and peptides (which are less strongly retained) gave elongated spots owing to the formation of a very irregular front or of a double front in the presence of tetrahydrofuran.

Fig. 2 shows the separations of ten glycyl dipeptides and twelve amino acids on

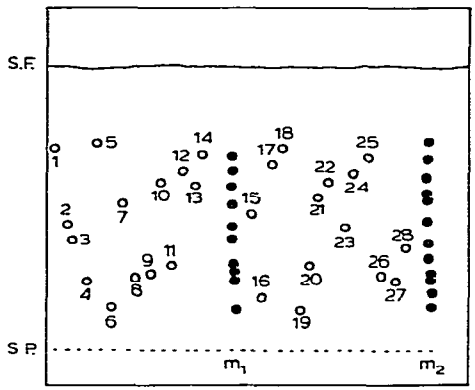

Fig. 2. Separations of dipeptides and amino acids on RP-18 plates impregnated with 4% HDBS using 1 *M* acetic acid + 0.2 *M* HCl in methanol–water (50:50) as mobile phase. Migration distance: 7.5 cm. Development time: 60 min. S.P. = Start point; S.F. = solvent front. Spots: 1 = Gly-Thr; 2 = Gly-Val; 3 = Gly-Met; 4 = Gly-Trp; 5 = Gly-Glu; 6 = Gly-His; 7 = Gly-Pro; 8 = Gly-Phe; 9 = Gly-Leu; 10 = Gly-Ala; 11 = Gly-Ile; 12 = Gly-Gly; 13 = Gly-Tyr; 14 = Gly-Ser; m_1 = mixture of 2, 3, 4, 5, 7, 9, 10, 11, 12 and 14; 15 = Pro; 16 = Trp; 17 = Thr; 18 = Glu; 19 = His; 20 = Ile; 21 = Tyr; 22 = Ala; 23 = Val; 24 = Gly; 25 = Ser; 26 = Leu; 27 = Phe; 28 = Met; m_2 = mixture of 15, 16, 17, 18, 19, 20, 21, 22, 23, 26, 27 and 28.

RP-18 plates eluted with 1 *M* acetic acid + 0.2 *M* hydrochloric acid in 50% methanol.

These impregnated layers, therefore, exhibit many advantages over the home-made ones, having greater sensitivity and, overall, the ability to separate a higher number of compounds in a single elution.

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