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SOAP THIN-LAYER CHROMATOGRAPHY OF SOME PRIMARY ALIPHATIC AMINES

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SUMMARY

The chromatographic characteristics of nine primary aliphatic amines have been studied using soap thin-layer chromatography. The influence of the type of detergent and its concentration, of the organic solvent and of the acid concentration on the chromatographic behaviour of these amines was investigated. Many separations that cannot be effected with either ion-exchange or reversed-phase chromatography have been effected.

INTRODUCTION

The use of detergents in column chromatography (soap chromatography)[1] has recently been developed and seems to offer interesting possibilities for analytical applications. As thin-layer chromatography (TLC) is also of great importance, we have tried to transfer soap chromatography from columns to thin layers in order to ascertain whether results as good as or better than those on ion exchangers could be achieved.

This first paper concerns some primary amines that we have already studied on ion exchangers[2] and to which Knox and Jurand[3] have applied soap column chromatography in order to investigate both the analytical applications and the differences in the chromatographic behaviours of these compounds with respect to column chromatography and ion-exchange TLC.

EXPERIMENTAL

The solutions were obtained by dissolving the amines in 0.01 *M* hydrochloric acid in a 1:1 (v/v) mixture of water and methanol to give a concentration of 2 mg/ml. The amount of each amine deposited on the layer was 2 μg.

Detection

The amines were detected by spraying a solution of 1% ninhydrin in a 5:1 (v/v) mixture of pyridine and glacial acetic acid and then heating the layers at 100° for 5 min.

Preparation of the layers

The layers for soap chromatography (thickness 300 μm) were prepared with a Chemetron automatic apparatus by mixing 12 g of silanized silica gel 60 HF (Merck, Darmstadt, G.F.R.) in 40 ml of 95% ethanol solution with a known concentration of detergent. Before use, the layers were dried at room temperature for 12 h. Sodium laurylether sulphate (LES) and triethanolamine dodecylbenzenesulphonate (DBS) were used as detergents. All of the measurements were carried out at 25°. The migration distance was 11 cm unless otherwise stated.

RESULTS AND DISCUSSION

In contrast to the results in soap column chromatography, where the detergent concentration in the eluent plays an important role[1,3], in soap TLC the detergent, if dissolved in the eluent, does not affect the chromatographic behaviour of the amines. Therefore, it is necessary to impregnate the silanized silica gel with the detergent as described under Experimental.

Influence of the type of detergent

The anionic detergents DBS and LES have very different chemical structures. On comparing the behaviour of the amines under the same experimental conditions on silica gel layers impregnated with these two detergents, it was found that the amines were retained more strongly on DBS than on LES, but the sequences of affinities were identical. For this reason, all of the data given here refer to layers impregnated with DBS.

Influence of the detergent concentration

Table I gives the R_F values of nine amines on thin layers of silanized silica gel containing increasing amounts of DBS. The DBS concentrations refer to the alcoholic solution in which the silanized silica gel was suspended when the layers were prepared. It should be noted that in the absence of detergent the amines were slightly retained, even if none ran with the solvent front. In every instance, however, very compact

TABLE I

R_F VALUES OF PRIMARY AMINES ON THIN LAYERS OF SILANIZED SILICA GEL ALONE (a) AND IMPREGNATED WITH 0.25% (b), 0.5% (c), 1% (d), 2% (e), 3% (f) AND 4% (g) DBS SOLUTION

Eluent: water–methanol–acetic acid (54.3:40:5.7).

No.	*Amine*	*(a)*	*(b)*	*(c)*	*(d)*	*(e)*	*(f)*	*(g)*
1	Octopamine	0.92	0.81	0.80	0.63	0.41	0.37	0.38
2	Amphetamine	0.67	0.53	0.42	0.30	0.16	0.11	0.09
3	Histamine	0.90	0.72	0.60	0.36	0.15	0.10	0.07
4	Tryptamine	0.67	0.49	0.38	0.22	0.15	0.10	0.09
5	3-Hydroxytyramine	0.84	0.79	0.72	0.60	0.43	0.34	0.36
6	Tyramine	0.80	0.71	0.62	0.48	0.35	0.26	0.26
7	1-Phenylethylamine	0.70	0.53	0.42	0.30	0.18	0.12	0.12
8	2-Phenylethylamine	0.71	0.55	0.42	0.27	0.16	0.10	0.09
9	Noradrenaline	0.83	0.83	0.78	0.69	0.54	0.46	0.48

spots were obtained, in contrast to column chromatography where, in the absence of detergent, the amines were not retained, giving rise to wide elution peaks[3].

As the concentration of the DBS solution is increased, the retention increases until a DBS concentration between 2 and 3% is reached, the R_F values of most amines becoming constant at higher DBS concentrations, and the affinity sequence is reversed for some amines. The constancy of the R_F values for DBS concentrations above 3% can be ascribed to saturation of the layer, similar to the effect observed in column chromatography[3], where such saturation is reached at a detergent concentration in the eluent of about 0.05%. The reversal of the R_F sequence, which has also been found in column chromatography, seems to be correlated with the structure of the amines. In fact, tryptamine, which differs from the other amines in having an indole nucleus, exhibits an affinity towards the detergent such that it is the most retained on silica gel impregnated with 4% of DBS, notwithstanding its high R_F value in the absence of detergent.

The presence in the molecule of one or more hydroxyl groups greatly affects the selectivity of the detergent towards the amines, the R_F values increasing in the following sequence: $C_6H_5 \cdot CH_2 \cdot CH_2NH_2$, 0.10; 4-OH$\cdot C_6H_5 \cdot CH_2 \cdot CH_2NH_2$, 0.26; 3,4-di-OH$\cdot C_6H_5 \cdot CH_2 \cdot CH_2NH_2$, 0.34; and 3,4-di-OH$\cdot C_6H_5 \cdot CH(OH) \cdot CH_2NH_2$, 0.46. This above-mentioned sequence refers to layers impregnated with 3% DBS solution. The length and the branching of the side-chain, however, have only a slight effect on the chromatographic behaviour of the amines (see 1-phenylethylamine, 2-phenylethylamine and amphetamine).

Influence of the methanol concentration

The presence of methanol in the eluent and its influence on the chromatographic behaviour of the amines was studied on layers impregnated with 2% DBS solution. A constant amount of glacial acetic acid was added to the eluent in order to have the amines in the protonated form so that interactions with the functional groups of the detergent could be established. The R_F trends for some amines with increasing percentages of methanol in the eluent are reported in Fig. 1. It should be noted that only the 20–80% range was considered, as in soap chromatography with methanol concentrations below 20% the eluent runs very irregularly.

Fig. 1 shows that an increase in the methanol concentration from 20 to 40% does not result in marked differences in the retention of most of the amines. For methanol concentrations above 40%, however, there is a considerable increase in the R_F values, which tend towards 0.6–0.7 for all amines at a methanol concentration of about 80%.

The optimal separation of the amines was obtained with methanol concentrations in the eluent between 30 and 40%. The decrease in the retention of the amines with an increase in the concentration of methanol in the eluent agrees with that predicted from reversed-phase chromatography. On plotting R_M as a function of the concentration of methanol in the eluent, trends similar to those reported in Fig. 1 are obtained, the only difference being the direction of curvature. In column chromatography, on the other hand, on plotting k values as a function of the concentration of organic solvent almost straight lines are obtained. The retention of the amines is much stronger on thin layers than on columns as organic solvent concentrations between 10 and 30% are used in column chromatography[3].

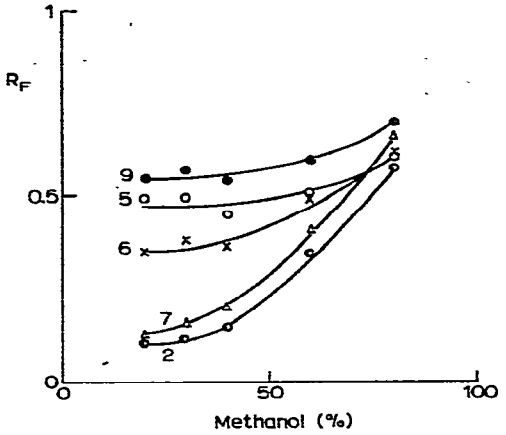


Fig. 1. R_F values *versus* concentration of methanol in the eluent for some amines on thin layers of silanized silica gel impregnated with 2% DBS solution. Eluent: water–acetic acid (5.7%)–methanol. Curves: 2, amphetamine; 7, 1-phenylethylamine; 6, tyramine; 5, 3-hydroxytyramine; 9, noradrenaline.

Influence of the acidity

The acidity was changed by adding increasing amounts of hydrochloric acid to the eluent, keeping the concentrations of glacial acetic acid and methanol constant. In the presence of hydrochloric acid, the formation on the layer of a double front, which is hardly perceptible in its absence, can be observed. The R_F value of the first front corresponds to that of octopamine. The R_F values of the nine amines at different hydrochloric acid concentrations are reported in Table II. As the acid concentration is increased, an increase of the R_F values is observed and this effect seems to be correlated with the presence of an ion-exchange mechanism in the chromatographic process.

On plotting the R_M values as a function of the apparent pH of the eluent, curves are obtained that approach linearity only for some of the amines. Their slopes, however, are between 0.3 and 0.4, which is much smaller than that expected if only

TABLE II

R_F VALUES OF PRIMARY AMINES ON THIN LAYERS OF SILANIZED SILICA GEL IMPREGNATED WITH 2% DBS SOLUTION

Eluents: hydrochloric acid solutions in water–methanol (40%)–acetic acid (5.7%).

No.	*Amine*	*Concentration of hydrochloric acid (N)*			
		0.05	*0.1*	*0.25*	*0.5*
1	Octopamine	0.50	0.66	0.69	0.76
2	Amphetamine	0.25	0.30	0.32	0.42
3	Histamine	0.34	0.46	0.54	0.73
4	Tryptamine	0.20	0.25	0.26	0.39
5	3-Hydroxytyramine	0.50	0.61	0.61	0.68
6	Tyramine	0.41	0.50	0.52	0.56
7	1-Phenylethylamine	0.26	0.31	0.33	0.41
8	2-Phenylethylamine	0.25	0.30	0.32	0.42
9	Noradrenaline	0.57	0.65	0.67	0.68

an ion-exchange mechanism is operating[4]. The presence of a double front accounts only partly for the R_M *versus* pH trends; a marked influence, on the other hand, of the non-ionic interactions between the amines and the side-chain of DBS can be excluded, as such interactions are weaker than those found for the same amines on polystyrene-based exchangers (the R_F values of these amines are higher on DBS than on Dowex 50-X4)[2].

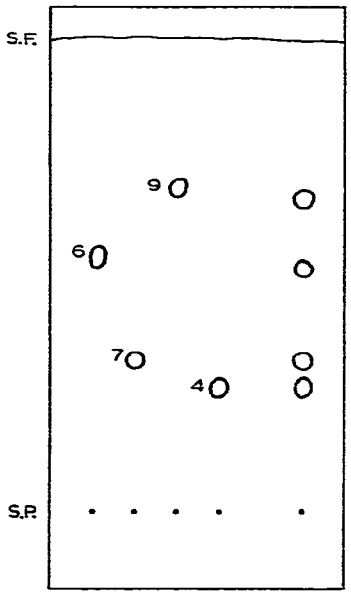
We can therefore conclude that the chromatographic behaviour of the nine amines can be ascribed to a partition process between the two phases rather than to an ion-exchange mechanism.

On columns the influence of acid concentration has been studied only superficially as mineral acid concentrations above about 10^{-2} *M* cause slow hydrolysis of silica-bonded alkyl groups.

Analytical applications

On the basis of the data in Tables I and II, many separations among the different amines can be effected; we carried out the separations shown in Figs. 2 and 3. The first was effected on layers impregnated with 2% and the second with 4% DBS solution.

It should be noted that the separation shown in Fig. 3 cannot be effected with lower DBS concentrations on the layer, indicating the possibility of obtaining different



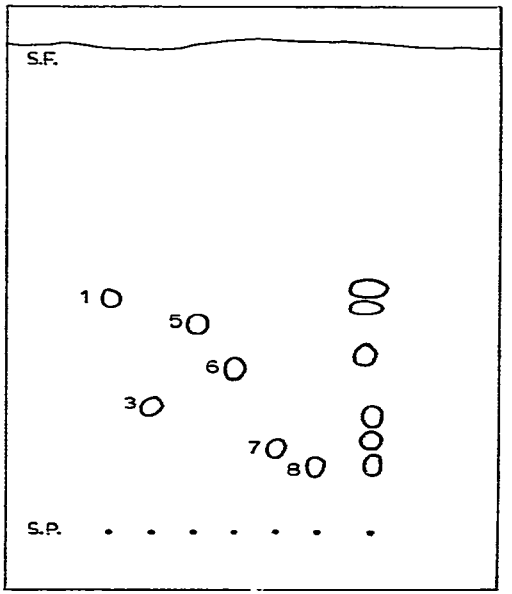


Fig. 2. Thin-layer chromatogram of some amines on silanized silica gel impregnated with 2% DBS solution. Migration distance: 12.5 cm. Eluent: 0.1 *M* hydrochloric acid solution in water–methanol (40%)–acetic acid (5.7%). Spots: 6, tyramine; 7, 1-phenylethylamine; 9, noradrenaline; 4, tryptamine.

Fig. 3. Thin-layer chromatogram of some amines on silanized silica gel impregnated with 4% DBS solution. Migration distance: 13 cm. Eluent: 0.1 *M* hydrochloric acid solution in water–methanol (40%)–acetic acid (5.7%). Spots: 1, octopamine; 3, histamine; 5, 3-hydroxytyramine; 6, tyramine; 7, 1-phenylethylamine; 8, 2-phenylethylamine.

separations simply by changing the amount of detergent on the layer. In comparison with ion-exchange chromatography[2], this technique permits the separation of a larger number of amines, as smaller differences in their R_F values are necessary and as the amines run much further and at different rates along the layer. By using two-dimensional TLC, as Fig. 4 shows, the separation of seven amines can be effected.

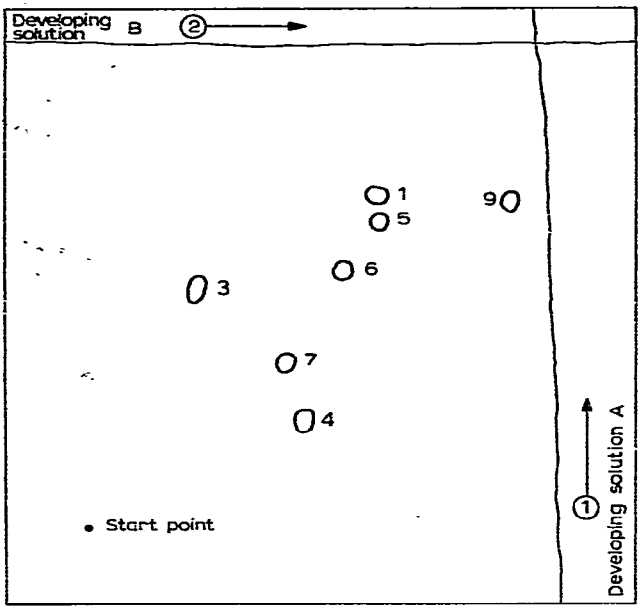


Fig. 4. Two-dimensional separation of seven amines on layers of silanized silica gel impregnated with 2% DBS solution. (1) Development with 0.1 *M* hydrochloric acid solution in water–methanol (40%)–acetic acid (5.7%) (solution A); (2) development with water–methanol–acetic acid (34.3:60:5.7) (solution B). Migration distance: 13 cm in the first direction and 11 cm in the other direction. Spots: 1, octopamine; 3, histamine; 4, tryptamine; 5, 3-hydroxytyramine; 6, tyramine; 7, 1-phenylethylamine; 9, noradrenaline.

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