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SOAP THIN-LAYER CHROMATOGRAPHY OF PRIMARY AROMATIC AMINES

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SUMMARY

The chromatographic behaviour of 35 primary aromatic amines was investigated by soap thin-layer chromatography, the amount of detergent on the layer and the concentration of methanol and hydrochloric acid in the eluent being varied. The R_M versus pH trends of the different amines on layers of silanized silica gel, alone and impregnated with 2% and 4% triethanolamine dodecylbenzenesulphonate solutions, are discussed. Several interesting separations that cannot be effected by either ion-exchange or reversed-phase chromatography were carried out.

INTRODUCTION

In a previous paper¹, the advantages of soap thin-layer chromatography (TLC) in the separation of some aliphatic amines were pointed out. We therefore considered it interesting, to study the behaviour of other compounds both in order to acquire a better knowledge of the retention mechanism and to establish possible analytical applications. We have studied primary aromatic amines so that their behaviour could be compared with that on thin layers of strong and weak anion and cation exchangers²⁻⁴.

EXPERIMENTAL

Solutions were obtained dissolving the amines in 0.05 M hydrochloric acid in a 1:1 (v/v) mixture of water and *n*-propanol to give a concentration of 2 mg/ml. The amount of each amine on the layer was between 0.5 and 2 μ g, except for *o*-bromoaniline, 2,4-dinitroaniline, 2,4-dichloroaniline and 2,6-dimethylaniline, the amount of which was 4 μ g. Fresh solutions were used for those amines which easily decompose (*o*- and *p*-phenylenediamine, 4-aminodiphenylamine and 4-amino-4'-methoxydiphenylamine).

The amines were detected with a solution of 5% N,N-dimethyl-*p*-aminobenzaldehyde in a 5:1 (v/v) mixture of ethanol and glacial acetic acid.

Preparation of the layers

The thin layers (thickness 300 μm) were prepared with a Chemetron automatic apparatus by mixing 20 g of silanized silica gel 60 HF (Merck, Darmstadt, G.F.R.) in 50 ml of a 95% ethanol solution containing a known concentration of detergent. Before use, the layers were dried at room temperature for 12 h.

Sodium laurylesulphate (LES), triethanolamine dodecylbenzenesulphonate (DBS) and sodium dodecylhydrogensulphate (DHS) were used as detergents. With DHS, the solid detergent was mixed directly with the silica gel at the time of preparation of the layers.

All the measurements were carried out at 25°. The migration distance was 11 cm unless stated otherwise.

RESULTS AND DISCUSSION

Among the three detergents used, the best results were achieved with DHS and DBS, which exhibited the same behaviour towards the amines. As the study with aliphatic amines¹ was effected on layers impregnated with DBS, we considered it useful to carry out this investigation with the same detergent so that the present results could be compared with the former results under the same experimental conditions.

Influence of detergent concentration

Table I gives the R_F values of 35 primary aromatic amines on silanized silica gel, alone and impregnated with increasing amounts of DBS. An aqueous-organic mixture of 1 *M* acetic acid and 30% methanol was used as the eluent.

On silanized silica gel layers the aromatic amines are generally substantially retained and exhibit a higher affinity towards the stationary phase than the catecholamines¹. On the layers impregnated with DBS, lower R_F values of the amines in comparison with those on the layers without detergent and, for some compounds, a reversal of the elution sequence can be observed. An increase in detergent concentration generally involves an increase in the retention by the layer; a limiting value, however, is not reached. Such behaviour disagrees with that observed with catecholamines, which therefore precludes the assumption that the attainment of a limiting R_F value for detergent concentrations of 3% and above with aliphatic amines¹ could be ascribed to saturation of the stationary phase by the detergent.

The influence of substituent groups on the chromatographic behaviour of the amines can be seen from Table I by reference to the behaviour of anilines. The introduction on to the ring of $-\text{CH}_3$, $-\text{OCH}_3$, $-\text{NO}_2$, $-\text{Br}$ and $-\text{Cl}$ groups involves an increase in the retention by the layer, whereas the opposite behaviour is observed on introduction of an $-\text{NH}_2$ group. The presence of two aromatic rings causes a considerable decrease in the R_F value. As regards the influence of the position of the substituent on the chromatographic behaviour of the aromatic amines, there are some differences on thin layers of silanized silica gel alone and impregnated with detergent. On the latter layer the *ortho*-isomers are less retained in the case of toluidines, anisidines and bromoanilines and more retained in the case of phenylenediamines, diaminotoluenes and nitroanilines. Such behaviour can be used as the basis for the separation of the isomers.

TABLE I

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

Eluent: water-methanol-acetic acid (64.3:30:5.7, v/v/v).

Amine	Thin layer					
	a	b	c	d	e	f
Aniline	0.70	0.52	0.42	0.30	0.21	0.17
<i>m</i> -Toluidine	0.64	0.38	0.31	0.22	0.14	0.11
<i>o</i> -Toluidine	0.65	0.43	0.36	0.27	0.19	0.15
<i>p</i> -Toluidine	0.64	0.38	0.32	0.22	0.14	0.11
2,4-Dimethylaniline	0.56	0.29	0.23	0.13	0.09	0.06
2,6-Dimethylaniline	0.44	0.31	0.27	0.18	0.13	0.10
<i>m</i> -Nitroaniline	0.41	0.36	0.36	0.35	0.29	0.28
<i>o</i> -Nitroaniline	0.32	0.29	0.27	0.26	0.23	0.22
<i>p</i> -Nitroaniline	0.39	0.36	0.36	0.35	0.30	0.30
2,4-Dinitroaniline	0.33	0.31	0.29	0.24	0.21	0.16
<i>m</i> -Bromoaniline	0.37	0.27	0.23	0.17	0.13	0.10
<i>o</i> -Bromoaniline	0.70	0.51	0.43	0.24	0.21	0.19
<i>p</i> -Bromoaniline	0.42	0.26	0.20	0.14	0.09	0.07
<i>p</i> -Chloroaniline	0.44	0.31	0.25	0.16	0.11	0.07
2,4-Dichloroaniline	0.19	0.16	0.15	0.13	0.09	0.07
<i>m</i> -Anisidine	0.62	0.40	0.35	0.24	0.18	0.14
<i>o</i> -Anisidine	0.64	0.43	0.37	0.28	0.21	0.16
<i>p</i> -Anisidine	0.70	0.45	0.37	0.25	0.18	0.14
<i>m</i> -Phenylenediamine	0.86	0.68	0.62	0.46	0.38	0.30
<i>o</i> -Phenylenediamine	0.79	0.58	0.47	0.35	0.28	0.22
<i>p</i> -Phenylenediamine	0.87	0.75	0.68	0.49	0.40	0.32
2,6-Diaminotoluene	0.85	0.61	0.58	0.41	0.31	0.22
2,4-Diaminotoluene	0.79	0.57	0.48	0.34	0.26	0.18
3,4-Diaminotoluene	0.69	0.43	0.35	0.22	0.16	0.11
3,4-Diaminonitrobenzene	0.58	0.52	0.50	0.44	0.39	0.33
α -Naphthylamine	0.38	0.24	0.17	0.12	0.07	0.05
4-Aminodiphenylamine	0.44	0.17	0.11	0.05	0.03	0.02
2-Aminodiphenylamine	0.22	0.11	0.08	0.04	0.02	0.01
3-Methoxy-4-aminodiphenylamine	0.38	0.15	0.08	0.04	0.02	0.01
4-Methoxy-4'-aminodiphenylamine	0.42	0.17	0.11	0.05	0.03	0.02
4,4'-Diaminodiphenylamine	0.84	0.48	0.32	0.13	0.06	0.04
2,4-Dinitro-4'-aminodiphenylamine	0.24	0.14	0.10	0.07	0.03	0.02
Benzidine	0.68	0.29	0.24	0.12	0.06	0.04
<i>o</i> -Tolidine	0.49	0.16	0.11	0.07	0.03	0.02
<i>o</i> -Dianisidine	0.29	0.14	0.10	0.08	0.05	0.04

Influence of percentage of methanol in the eluent

The aromatic amines behave in a similar manner to the catecholamines^{1,5} on an increase in the percentage of methanol in the eluent. On elution with a mixture containing 80% of methanol, the 35 amines exhibit R_F values between 0.67 and 0.80. The strongest resolution power of the layer is achieved at a low methanol content (30%).

Influence of acid concentration in the eluent

In order to study the influence of the acid concentration in the eluent on the

behaviour of the aromatic amines, we carried out measurements on layers of silanized silica gel alone and impregnated with 2% and 4% DBS, eluting with solutions with various hydrochloric acid concentrations in methanol-acetic acid-water mixtures. Fig. 1 shows the R_M versus apparent pH trends of the different amines on silanized silica gel layers, and the influence of the acidity in the absence of detergent can be seen.

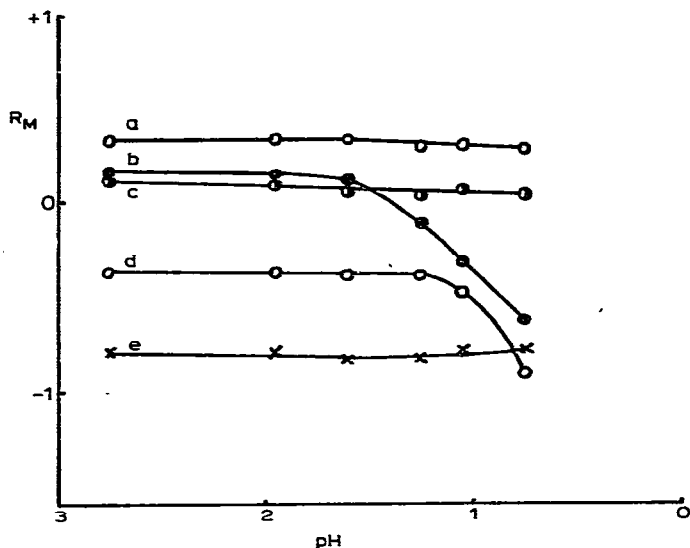


Fig. 1. R_M values versus apparent pH of the eluents for some aromatic amines on thin layers of silanized silica gel. Eluents: water-methanol (30%)-acetic acid (5.7%) and hydrochloric acid mixtures. (a) *o*-Nitroaniline; (b) *m*-nitroaniline; (c) 4-aminodiphenylamine; (d) aniline; (e) *m*-phenylenediamine.

Curve (a) is peculiar to those amines, such as *o*-nitroaniline and 2,4-dinitroaniline, which are completely deprotonated in the pH range studied. Their constant R_F values with changing pH indicate that changes in the protonic activity have no effect on their chromatographic behaviour, contrary to what would be expected. It should be noted, furthermore, that the behaviour of these amines is not correlated with the ionic strength of the eluent, as was shown by some experiments carried out on the same layers using sodium chloride solutions at concentrations between 0.025 and 0.25 *M* in an aqueous-organic mixture containing methanol (30%) and acetic acid (5.7%) as the eluent. Experiments at constant pH and changing ionic strength were also effected with amines in the protonated form, and it was found that even for these compounds the influence of the ionic strength is negligible.

Curves (c) and (e) relate to mono- and diamines that have at least one protonated amino group in the pH range studied. Also for these compounds constancy of the R_F values with varying pH was found. Curve (e) relates to compounds such as phenylenediamines, toluenediamines and 4,4'-diaminodiphenylamine and curve (c) to amines that exhibit a higher affinity towards the stationary phase, such as 2,4-dimethylaniline, 4-amino-diphenylamine and 3-methoxy-4-aminodiphenylamine. Curves (b) and (d) are peculiar to monoamines whose chromatographic behaviour

is affected by a change in the pH of the eluent, probably owing to the progressive protonation of their amino group.

It should be noted, however, that the influence of pH is perceptible only at low pH values. Such behaviour seems to be in disagreement with the acid-base characteristics of several amines (*m*- and *p*-nitroaniline, the three bromoanilines, *p*-chloroaniline and 2,4-dichloroaniline) whose chromatographic behaviour should be affected by a change in pH over the whole pH range studied as their pK_a values are between 1.00 and 3.98 (ref. 6). Such disagreement can be explained on the basis of the pH differences in the layer and in the solution. With pH measurements on the layer as described in a previous paper⁷, we obtained the trends shown in Fig. 2. Such trends, which relate to different pH values of the eluent, indicate the formation on the layers of a pH gradient, the start of which is increasingly delayed as the acid concentration in the eluent increases. In particular, the pH on the layer does not change appreciably with eluents with higher pH values [curves (a) and (b) in Fig. 2], notwithstanding the 0.8 pH unit difference. Such an occurrence accounts for the constancy of the R_F values in the pH range 2.75–1.95 for those amines which are not completely protonated.

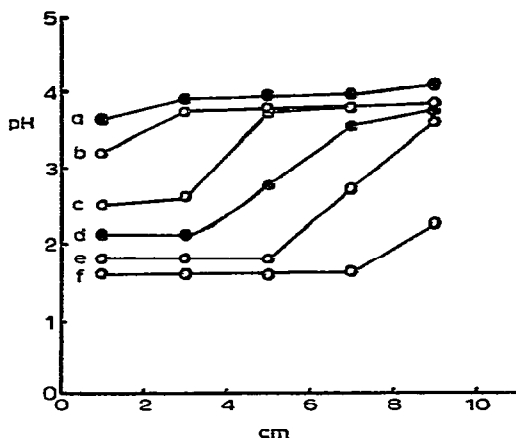


Fig. 2. pH values of silanized silica gel suspensions referred to the centre of the strips (2×10 cm)⁷. Apparent pH of the eluents: (a) 2.75; (b) 1.95; (c) 1.60; (d) 1.25; (e) 1.05; (f) 0.75.

The chromatographic characteristics of the amines on layers of silanized silica gel impregnated with 2% DBS solution are reported in Table II. Contrary to their behaviour on silanized silica gel alone, all of the amines exhibit increasing R_F values with a decrease in pH, except for *o*-nitroaniline and 2,4-dinitroaniline, whose R_F values do not change. The highest increase in the R_F values is observed with benzidine, *o*-tolidine, *o*-dianisidine and 4,4'-diaminodiphenylamine, probably owing to the presence in their molecules of two amino groups that are completely protonated at the pH values listed in Table II.

In order to illustrate the influence of the pH of the eluent on the behaviour of the amines on these layers, Fig. 3 shows some typical R_M versus pH trends. Comparison of these trends with those in Fig. 1 reveals a similarity in the pH range 2.75–1.95 and a marked difference at lower pH values.

TABLE II

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

Eluents: hydrochloric acid solutions in water-methanol (30%)–acetic acid (5.7%)

Amine	pH of eluent				
	1.95	1.60	1.25	1.05	0.75
Aniline	0.34	0.42	0.50	0.60	0.70
<i>m</i> -Toluidine	0.23	0.29	0.38	0.49	0.55
<i>o</i> -Toluidine	0.27	0.35	0.45	0.56	0.61
<i>p</i> -Toluidine	0.23	0.30	0.40	0.51	0.57
2,4-Dimethylaniline	0.16	0.21	0.25	0.34	0.45
2,6-Dimethylaniline	0.21	0.27	0.33	0.40	0.54
<i>m</i> -Nitroaniline	0.35	0.36	0.47	0.60	0.65
<i>o</i> -Nitroaniline	0.26	0.28	0.29	0.29	0.29
<i>p</i> -Nitroaniline	0.35	0.36	0.39	0.49	0.57
2,4-Dinitroaniline	0.24	0.25	0.24	0.24	0.25
<i>m</i> -Bromoaniline	0.17	0.21	0.29	0.40	0.46
<i>o</i> -Bromoaniline	0.24	0.28	0.43	0.57	0.65
<i>p</i> -Bromoaniline	0.15	0.19	0.25	0.35	0.43
<i>p</i> -Chloroaniline	0.18	0.22	0.30	0.40	0.49
2,4-Dichloroaniline	0.13	0.16	0.23	0.33	0.45
<i>m</i> -Anisidine	0.28	0.34	0.42	0.52	0.59
<i>o</i> -Anisidine	0.33	0.41	0.48	0.58	0.65
<i>p</i> -Anisidine	0.30	0.38	0.44	0.55	0.62
<i>m</i> -Phenylenediamine	0.53	0.60	0.69	0.76	0.80
<i>o</i> -Phenylenediamine	0.40	0.48	0.55	0.66	0.76
<i>p</i> -Phenylenediamine	0.58	0.67	0.75	0.80	0.82
2,6-Diaminotoluene	0.42	0.58	0.62	0.69	0.77
2,4-Diaminotoluene	0.38	0.49	0.56	0.66	0.76
3,4-Diaminotoluene	0.25	0.33	0.39	0.53	0.72
3,4-Diaminonitrobenzene	0.46	0.46	0.45	0.54	0.64
α -Naphthylamine	0.13	0.15	0.21	0.28	0.37
4-Aminodiphenylamine	0.07	0.09	0.13	0.18	0.28
2-Aminodiphenylamine	0.04	0.06	0.08	0.12	0.17
3-Methoxy-4-aminodiphenylamine	0.06	0.08	0.11	0.15	0.24
4-Methoxy-4'-aminodiphenylamine	0.07	0.09	0.13	0.20	0.36
4,4'-Diaminodiphenylamine	0.21	0.37	0.48	0.72	0.79
2,4-Dinitro-4'-aminodiphenylamine	0.08	0.09	0.12	0.17	0.26
Benzidine	0.15	0.27	0.45	0.62	0.80
<i>o</i> -Tolidine	0.08	0.16	0.31	0.51	0.76
<i>o</i> -Dianisidine	0.10	0.21	0.37	0.61	0.80

Particularly on layers impregnated with 2% DBS, the monoamines exhibit a rectilinear trend with a slope of about 0.6 in the pH range 1.60–0.75. The constancy of R_M values in the pH range 2.75–1.95 can be ascribed to the formation of a pH gradient similar to that observed with silanized silica gel [see curves (a) and (b) in Fig. 2], which involves small pH differences on the layer. The straight portion of a curve for an amine corresponds to the presence on the layer of an ion-exchange mechanism rather than a partition mechanism. The slope of the straight portions, which is lower than the theoretical value¹, can be ascribed to the formation of the pH gradient on the layer, and also to the presence of a partition process due to

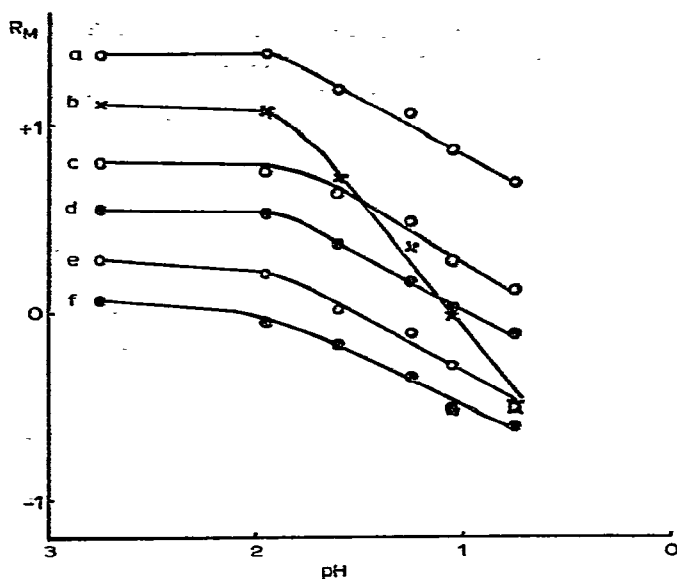


Fig. 3. R_M values versus apparent pH of the eluents for some aromatic amines on thin layers of silanized silica gel impregnated with 2% DBS solution. Eluents: water-methanol (30%)–acetic acid (5.7%) and hydrochloric acid mixtures. (a) 2-Aminodiphenylamine; (b) *o*-toluidine; (c) *p*-bromoaniline; (d) *p*-toluidine; (e) 2,4-diaminotoluene; (f) *m*-phenylenediamine.

the use of aqueous-organic eluents. Such an occurrence concerns those amines whose R_F values at a given pH of the eluent are higher than that at the beginning of the gradient. Therefore, the behaviour of some diamines, such as the phenylenediamines and toluenediamines, which are less affected than the other diamines by changes in the pH of the eluent, can be ascribed to their R_F values being higher than that at the beginning of the gradient; the pH gradient on the layer, in fact, involves the incomplete protonation of the two amino groups and therefore slopes similar to those for the monoamines are achieved [see curves (e) and (f) in Fig. 3]. The diamines, which exhibit R_F values lower than that at the beginning of the gradient, give rise to straight portions with a slope twice that of the monoamines [see curve (b) in Fig. 3].

As the detergent concentration on the layer is increased (to 4%), R_M versus pH trends similar to those in Fig. 3 are obtained. A greater change with a decrease in the pH of the eluent in the pH range 2.75–1.95 and a higher slope of the straight portion below pH 1.95 are obtained. Such differences can be ascribed to the strongest retention of the amines as the detergent concentration increases and therefore R_F values smaller than that of the beginning of the pH gradient on the layer can be achieved.

o-Nitroaniline and 2,4-dinitroaniline exhibit constant R_F values over the whole pH range, even on layers impregnated with 4% DBS. Such values, however, are lower than those on 2% DBS and on silica gel alone [see columns (a), (d) and (f) in Table I]. The behaviour of these two amines, which are completely deprotonated over the whole pH range and therefore cannot give rise to an ion-exchange process, can be ascribed to an adsorption process by the hydrophobic side-chain of the detergent.

A similar behaviour has been observed for these compounds, although more marked, on polystyrene-based ion exchangers³, which confirms the similarity between soap and ion-exchange chromatography. This similarity has also been observed in the paper chromatography of some dyes⁸ and, more recently, in the column chromatography of amino acids⁹.

Analytical applications

Among all the separations that can be effected on the basis of the R_f values of the amines reported in Tables I and II and of those on layers impregnated with 4% DBS on changing the pH of the eluent, we carried out some of those which cannot be obtained on layers of polystyrene³ or cellulose-based² ion exchangers.

Fig. 4 shows the separation of the three phenylenediamines and that of the three nitroanilines and 2,4-dinitroaniline on silanized silica gel impregnated with 2% DBS, with 0.5 *M* hydrochloric acid in an aqueous-organic mixture containing 30% of methanol and 5.7% of acetic acid (pH = 1.05) as the eluent. On the same layers, the three toluenediamines have also been separated, with 0.1 *M* hydrochloric acid in the above aqueous-organic solution (pH = 1.60) as the eluent.

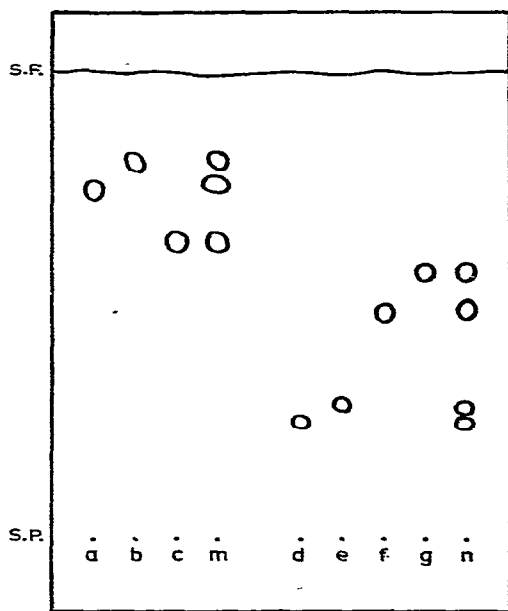


Fig. 4. Thin-layer chromatogram of some aromatic amines on silanized silica gel impregnated with 2% DBS solution. Migration distance: 12.5 cm. Eluent: 0.5 *M* hydrochloric acid solution in water-methanol (30%)-acetic acid (5.7%). (a) *m*-Phenylenediamine; (b) *p*-phenylenediamine; (c) *o*-phenylenediamine; (m) mixture of phenylenediamines; (d) 2,4-dinitroaniline; (e) *o*-nitroaniline; (f) *p*-nitroaniline; (g) *m*-nitroaniline; (n) mixture of dinitro- and nitroanilines.

By using the two-dimensional technique, we achieved the separation of 13 aromatic amines (see Fig. 5), eluting in the first direction with 1 *M* acetic acid in an aqueous-organic mixture containing 40% of methanol and in the second direction with the same eluent used in the separation of the amines in Fig. 4. On silica

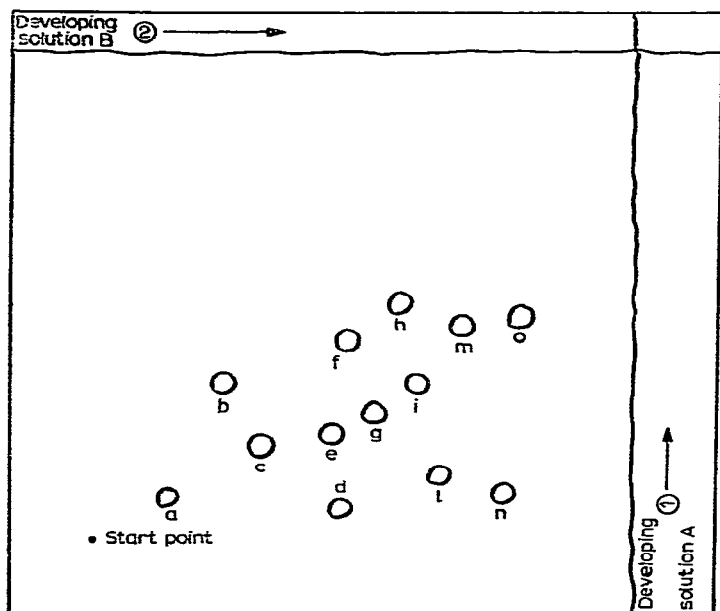


Fig. 5. Two-dimensional separation of 13 aromatic amines on layers of silanized silica gel impregnated with 2% DBS solution. (1) Development with water-methanol-acetic acid (54.3:40:5.7) (solution A); (2) development with 0.5 M hydrochloric acid solution in water-methanol (30%)-acetic acid (5.7%) (solution B). Migration distance: 13 cm in both directions. (a) 3-Methoxy-4-aminodiphenylamine; (b) 2,4-dinitroaniline; (c) α -naphthylamine; (d) *o*-toluidine; (e) *p*-bromoaniline; (f) *p*-nitroaniline; (g) *p*-toluidine; (h) 3,4-diaminotoluene; (i) *p*-anisidine; (l) benzidine; (m) aniline; (n) 4,4'-diaminodiphenylamine; (o) *p*-phenylenediamine.

gel, both alone and impregnated with the detergent, the separation of benzidine, *o*-toluidine and *o*-dianisidine and that of 4-aminodiphenylamine, 2-aminodiphenylamine and 4,4'-diaminodiphenylamine can also be carried out.

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