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THIN-LAYER CHROMATOGRAPHY OF SOME METHYLATED AMINO ACIDS WITH AQUEOUS SALT SOLUTIONS USED AS ELUENTS

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SUMMARY

The retention order of methylated lysine and arginine derivatives followed a reversed-phase separation pattern on various silicas with aqueous salt solutions used as eluents. Principal component analysis established that the origin of the silica exerts a dominant influence on their chromatographic performance, and the effect of impregnation and salt quality are of secondary importance. Good linear correlations were found between the lipophilicity-decreasing effect of monovalent cations and their ionic radii.

INTRODUCTION

Methylated amino acids play a considersble role in biological systems[1]; their determination is therefore of paramount importance. As methylation increases the lipophilicity of amino acids[2], and unmethylated amino acids have R_F values higher than 0.75 in thin-layer chromatography (TLC) with aqueous eluents[3,4], it was reasonable to assume that the separation of methylated amino acids could be carried out by reversed-phase TLC.

Recent research indicates that the main distinction between normal-phase and reversed-phase TLC is the relative polarity of the mobile and stationary phase[5]; in reversed-phase TLC the stationary phase has to be less polar than the mobile phase. This implies that the impregnation or chemical bonding of hydrophobic substituents to the support is not a prerequisite of reversed-phase TLC. The validity of this theory was tested and found to be true for some 3,5-dinitrobenzoate esters[6].

It is well known that the ions modify the partition of polar compounds between the aqueous and organic phases[7,8]. Although the underlying molecular processes are still a matter of discussion, this phenomenon has been frequently exploited in reversed-phase TLC to improve the separation of polar compounds[9–12]. The objectives of our work were to study the reversed-phase TLC behaviour of some methylated amino acids with various aqueous salt solutions on impregnated and on unimpregnated silica plates.

EXPERIMENTAL

The following silica plates were applied in our investigations: DC Alufolien

Kieselgel 60 (Merck, Darmstadt, F.R.G.); Polygram Sil G (Macherey-Nagel, Düren, F.R.G.); Silpress (Labor MIM, Budapest, Hungary); Silufol (Kavalier, Czecho-slovakia); Silufol, impregnated (Kavalier); hand-made layers of 0.25 mm thickness (Kieselgel 60, Merck); hand-made layers of 0.25 mm thickness, impregnated (Kie-selgel 60, Merck). Impregnation with 5% paraffin oil in *n*-hexane was carried out as described in ref. 13.

    Lysine (L), formyllysine (FL), mono-, di- and trimethyllysine (MML, DML and TML), arginine (A), mono-, di- and trimethylarginine (MMA, DMA and TMA) were dissolved in water–2-propanol (4:1) at a concentration of 1 mg/cm^3; 3 mm^3 of each solution were spotted on the plates. Aqueous solutions of lithium chloride, sodium chloride, potassium chloride, rubidium chloride, caesium chloride, sodium acetate, potassium bromide, potassium iodide, potassium nitrate and potassium sul-phate were applied as eluents in the concentration range of 0–1 *M*.

    After development the plates were dried at 105°C and the spots were detected by the ninhydrin reagent[14].

    To compare the retention characteristics of sorbents, taking into simultaneous consideration the R_F values of all amino acid derivatives, principal component analy-sis[15] was applied. The plates listed above (except impregnated Silufol) served as vari-ables, and the R_F values of amino acid determined at 0, 0.05, 0.1, 0.25 and 0.5 *M* sodium chloride concentrations served as observations. To compare the effect of sodium chloride and sodium acetate the R_F values determined on Silpress plates in eluents containing identical concentrations of sodium acetate were included in prin-cipal component analysis. Non-linear mapping of principal component loadings and variables was also carried out[16].

    To elucidate the effect of various cations (anion always chloride) on the reten-tion behaviour of MML, DML, TML, MMA and DMA, linear correlations were calculated between the corresponding R_M values and the ionic radii[17] of cations for Silufol and impregnated Silufol:

$$R_M = a + br_i \tag{1}$$

where R_M is the actual R_M value of an amino acid derivative determined in the presence of a cation of radius r_i (ionic concentration always 0.025 *M*), and r_i is the ionic radius of the monovalent cation.

RESULTS AND DISCUSSION

    The dependence of the retention of lysine derivatives on the salt concentration of eluent on different sorbents is shown in Figs. 1–7. The retention order of derivatives is identical in each case (FL < L < MML < DML < TML), indicating that the sorbents exhibit fairly similar adsorptive characteristics. The more lipophilic deriv-atives are retained more strongly, which means that the sorbents behave as reversed--phase TLC sorbents without any impregnation. This finding supports entirely the theory outlined in ref. 5, that the aqueous salt solutions are more polar than the adsorptive sites of silica, resulting in reversed-phase separation. The dependence of methylated derivatives on the salt concentration of the eluent is generally of satu-ration character; however, the exact shape of the curve differs from sorbent to sor-bent. The salts exert a negligible influence on the retention of formyllysine, but this
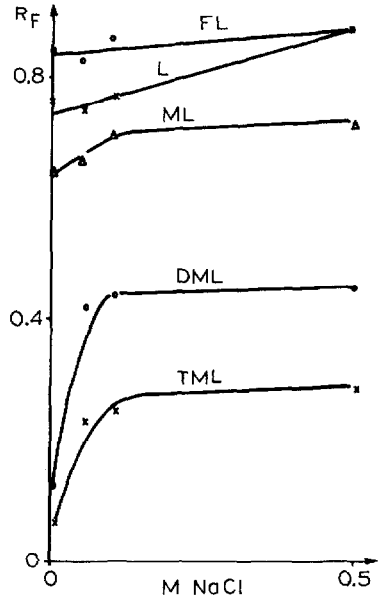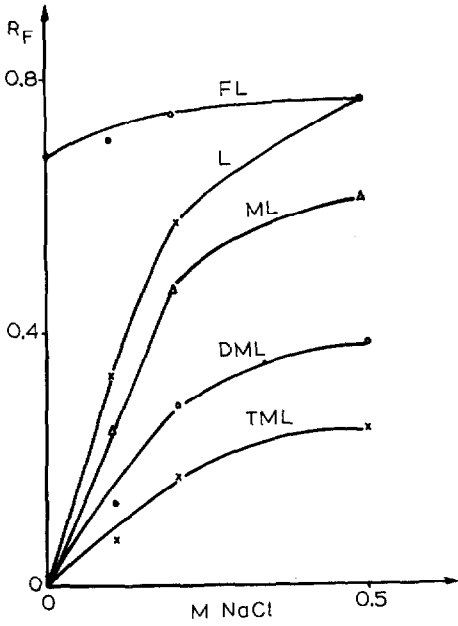
Fig. 1. Effect of sodium chloride on the retention of lysine derivatives (DC Alufolien Kieselgel 60, Merck).

Fig. 2. Effect of sodium chloride on the retention of lysine derivatives (Polygram Sil G, Macherey-Nagel).
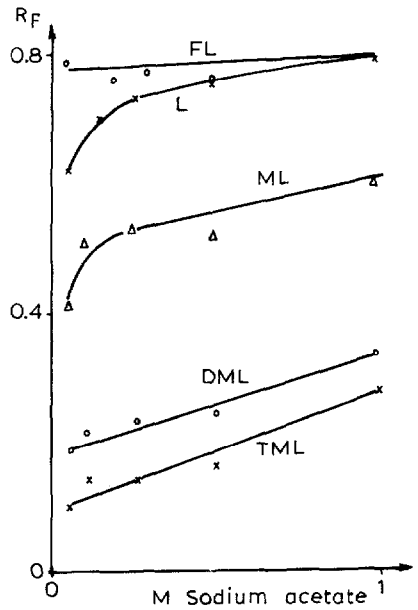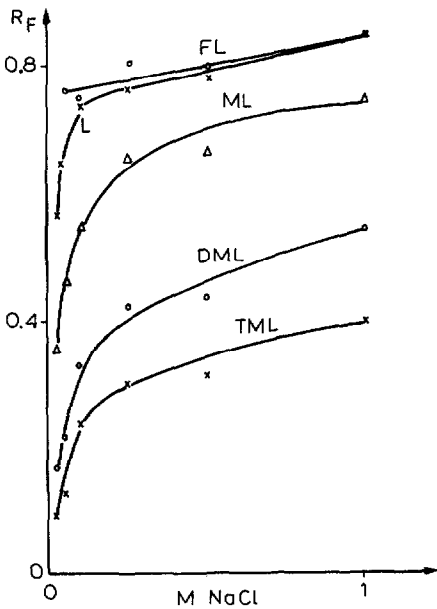

Fig. 3. Effect of sodium chloride on the retention of lysine derivatives (Silpress, Labor MIM, Hungary).

Fig. 4. Effect of sodium acetate on the retention of lysine derivatives (Silpress, Labor MIM, Hungary).
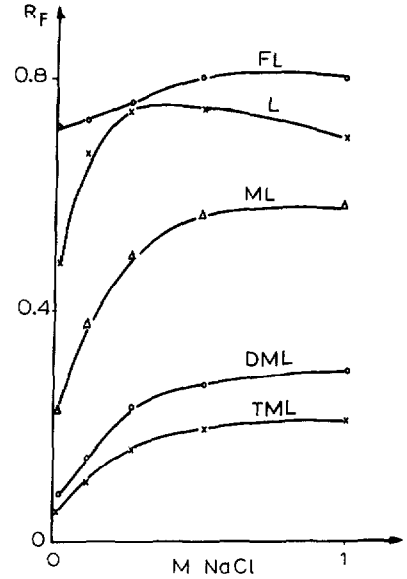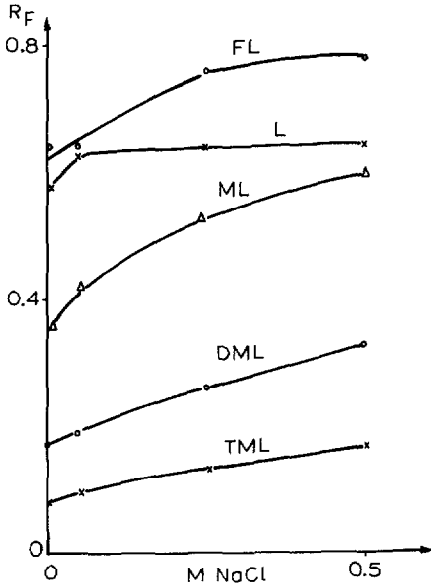
Fig. 5. Effect of sodium chloride on the retention of lysine derivatives (Silufol, Kavalier, Czechoslovakia).

Fig. 6. Effect of sodium chloride on the retention of lysine derivatives (hand-made silica plate).
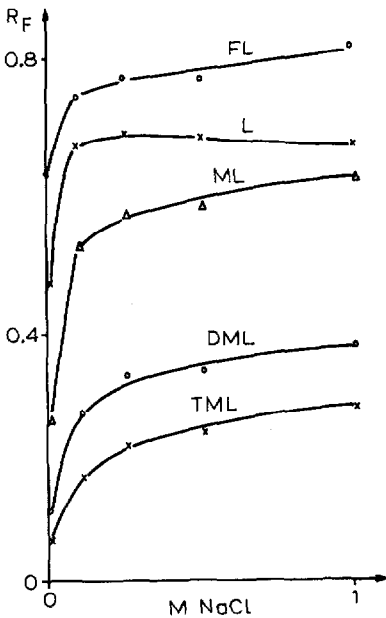

Fig. 7. Effect of sodium chloride on the retention of lysine derivatives (hand-made silica plate, impregnated).

observation may be due to the fact that the formyllysine exhibits very low retention soon in distilled water as eluent. On Kieselgel 60 plates only formyllysine shows significant mobility in distilled water (Fig. 1). The other compounds remain on the start. The R_F values increase monotically but not linearly with growing salt concentration of the eluent. On Polygram Sil G plates (Fig. 2) the retention pattern is different: the lysine derivative also show measurable mobility in distilled water, but over a certain limit the higher salt concentrations do not increase the mobility. On Silpress plates the effects of sodium chloride and sodium acetate differs considerably (Figs. 3 and 4); the effect of sodium chloride is greater and the retention curves differ markedly from each other. This phenomenon may be due in part to two facts: (i) the dissociation of sodium acetate results in a lower pH value of the eluent, which can influence the lipophilicity of the methylated amino acid derivatives; (ii) the acetate anion has a lower energy of hydration (-243.2 kJ/mol) than the chloride ion, therefore its salting-out effect is also lower[18].

Unlike the other sorbents, the retention dependence of methylated lysine derivatives on the sodium chloride concentration is quasi-linear on Silufol plates (Fig. 5). Impregnation exerts limited influence on the retention of formyllysine and lysine (Figs. 6 and 7), but decreases the retention of methylated derivatives. This observation suggests that the paraffin oil covers some adsorptive sites on the silica surface, resulting in enhanced mobility of solutes. The results of principal component analysis are summarized in Table I and in Figs. 8 and 9.

A single background component accounts for more than 86% of the total variance (Table I), *i.e.* all the sorbents investigated show similar retention patterns taking into consideration the retention behaviour of all amino acids. Fig. 8 clearly demonstrates that the character of silica has the greatest impact on the separation, Kieselgel 60 (point 1), Polygram Sil G (point 2), Silpress (point 3) and Silufol (point 4) plates are placed at the opposite ends of the map. The impregnation (points 4 and 5) and the type of salt (points 3 and 6) also influence the retention characteristics of silica, although to a lesser extent than its origin.
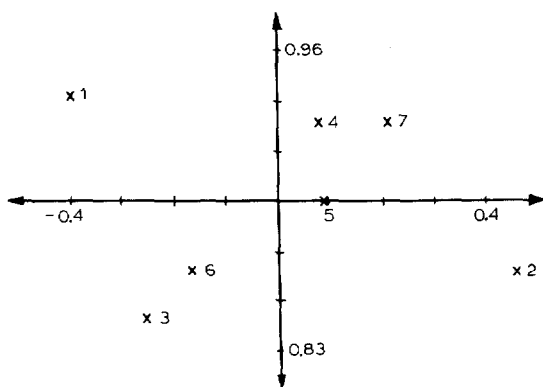

Fig. 8. Two-dimensional non-linear mapping of PC loadings. Number of iterations, 3; error of the mapping, $4.50 \cdot 10^{-2}$. Points: 1 = Kieselgel 60, Merck; 2 = Polygram Sil G; 3 = Silpress; 4 = hand-made plate; 5 = hand-made plate, impregnated; 6 = Silpress; 7 = Silufol. Eluent, sodium chloride (except 6, sodium acetate).
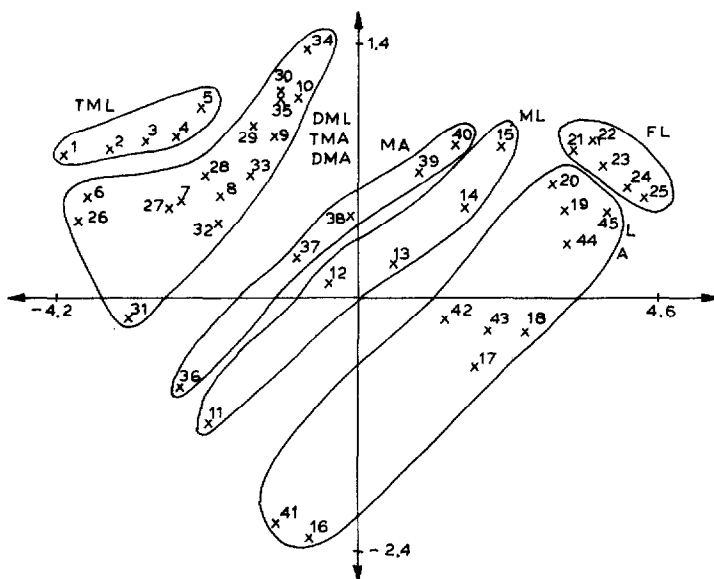
Fig. 9. Two-dimensional non-linear mapping of PC variables. Number of iterations, 7; error of the mapping, $1.74 \cdot 10^{-3}$. Points: 1–5 = TML; 6–10 = DML; 11–15 = MML; 16–20 = L; 21–25 = FL; 26–30 = TMA; 31–35 = DMA; 36–40 = MMA. Numbers indicate increasing salt concentration of the eluent.

The clustering of amino acids on the two-dimensional non-linear mapping of principal component variables indicates the difficulties in the separation of methylated amino acid derivatives. The compounds exhibiting very similar retention characteristics are very near to each other on the map they form common cluster. As FL, MML, TML and MMA form distinct clusters their separation can be easily carried out. Lysine and arginine form a common cluster; however, the area of the cluster is great therefore it is possible to find conditions that are adequate for their separation. The critical point is the common cluster of DML, DMA and TMA. Its existence suggests that separation of these three compounds is fairly difficult, and can be achieved only under rigorously controlled and specific conditions. However, when it is achieved, the same system will probably separate the other amino acid derivatives, also.

The parameters of eqn. 1 are listed in Table II. Monovalent cations with larger

TABLE I

RESULTS OF PRINCIPAL COMPONENT ANALYSIS

No. of component	Eigenvalue	Sum of total variance explained (%)
1	6.27	86.62
2	0.49	96.64
3	0.13	98.50

TABLE II

PARAMETERS OF LINEAR CORRELATIONS BETWEEN THE LIPOPHILICITY (R_M) OF AMINO ACID DERIVATIVES AND THE IONIC RADIUS (r_i)

Salt concentration, 0.025 M

$R_M = a + br_i$

$r_{99\%} = 0.9587$ $r_{99.9\%} = 0.9912$

Compound	a	b	s_b	$r_{calc.}$
Silufol support				
TML	1.78	−0.40	0.05	0.9768
DML	1.67	−0.62	0.10	0.9648
MML	0.66	−0.29	0.03	0.9856
DMA	0.85	−0.20	0.03	0.9686
MMA	0.65	−0.21	0.02	0.9890
Impregnated Silufol support				
TML	1.82	−0.39	0.04	0.9855
DML	1.37	−0.36	0.02	0.9967
MML	0.80	−0.34	0.02	0.9937
DMA	1.10	−0.25	0.02	0.9940
MMA	0.89	−0.30	0.02	0.9958

ionic radii exert greater effects on the lipophilicity of methylated amino acids. The ions probably suppress the dissociation of the highly polar carboxyl group, resulting in increased lipophilicity. This effect depends linearly on the ionic radius. The difference observed between the influence of sodium and potassium have some biological importance: it indicates that the equilibrium between Na^+ and K^+ influences not only the membrane phospholipids[19] but also the methylated amino acids present in biologically important proteins.

    The effects of various anions do not differ as much as those of the monovalent cations. This means that the anions also play a role in the determination of mobility of amino acids (perhaps by modifying the hydrate shell around them[20]); however, this effect is lower than that of the cations.

TABLE III

EFFECT OF VARIOUS ANIONS ON THE LIPOPHILICITY OF SOME METHYLATED AMINO ACIDS

Cation, K^+; quoted values are $100 \cdot R_M$; support, impregnated Silufol.

Anion		Amino acid				
Type	Molality	TML	DML	MML	DMA	MMA
Cl^-	0.025	140	97	44	83	59
Br^-	0.025	124	83	31	74	44
I^-	0.025	133	91	41	81	54
NO_3^-	0.025	152	106	49	87	61
SO_4^{2-}	0.0125	118	73	21	63	35

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