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THIN-LAYER CHROMATOGRAPHY OF SULPHONAMIDES ON SILICA GEL G AND POLYAMIDE LAYERS BY MEANS OF A POLAR MOBILE PHASE

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SUMMARY

A thin-layer chromatographic system based on polyamide or silica gel G layers and an aqueous mobile phase was used for the separation of a series of sulphonamides. A linear relationship between R_M values on polyamide layers and the acetone concentration in the mobile phase allowed the calculation of extrapolated R_M values. The influence of the nature of the stationary phase on the migration of compounds is discussed.

INTRODUCTION

Reversed-phase thin-layer chromatography (TLC) has been used to obtain R_M values of penicillins, cephalosporins and sulphonamides as an indication of their lipopholic character[1–3]. For penicillins and cephalosporins, the R_M values were shown to be significantly correlated with antibacterial activity[4]. The non-polar stationary phase used was a silica gel G layer impregnated with silicone oil DC 200; the polar mobile phase was a veronal acetate buffer at pH 7.4, alone or in mixtures with acetone[1–3].

However, it was considered interesting to find a TLC system that would provide R_M values better related to biological activity, for example by a more general study of the influence of the nature of stationary phases on the migration of compounds. In this paper are described studies with TLC systems in which the stationary phase was unimpregnated silica gel G, microcristalline cellulose, polyamide or Kieselguhr G and the mobile phase was veronal acetate buffer at pH 7.4, alone or in mixtures with acetone. In some experiments, the polyamide stationary phase was impregnated with silicone oil and other lipophilic compounds in order to compare the R_M values of sulphonamides obtained in these systems with those previously measured on impregnated silica gel G[3]. Although this work was not intended to be an investigation of TLC separation methods for sulphonamides, which have been dealt with in several publications[5–8], it showed the usefulness of an aqueous mobile phase in separating such compounds.

MATERIALS AND METHODS

Silica gel G (30 g) or Polyamide 11 (9 g) were stirred with 60 ml of water or 55 ml of ethanol, respectively, in order to coat five glass plates (20 × 20 cm)[9]. The polyamide layers were dried at room temperature and the silica gel G layers in an oven at 100° for 1 h. The mobile phase in both cases was veronal acetate buffer at pH 7.4, alone or mixed with different amounts of acetone. The compounds were dissolved in water, methanol or acetone (3 mg/ml) and 1 μl of solution was spotted on the plates. Two plates were developed simultaneously in a chromatographic chamber containing 200 ml of mobile phase. The developed plates were dried and sprayed with p-dimethylaminobenzaldehyde (0.1 % in ethanol)–concentrated hydrochloric acid (99:1)[10]. Yellow spots on a white background appeared.

The R_M values were calculated by means of the equation

$$R_M = \log \left\{ \frac{1}{R_F} - 1 \right\}$$

Stationary phases of different types were obtained by stirring 12 g of microcrystalline cellulose or 30 g of Kieselguhr G with 56 or 60 ml of water, respectively.

In some experiments, the polyamide layers were impregnated with silicone DC 200 (350 cS), squalane, undecane or liquid paraffin. The impregnation was carried out by developing the plates in a 5, 10 or 20% solution of silicone, squalane, undecane or liquid paraffin in diethyl ether or light petroleum (b.p. 40–60°)[3]. Eight plates were developed for 12–16 h in a single chromatographic chamber containing 200 ml of impregnating solution[1]. The presence of silicone oil was shown by means of an ether extraction. All of the adsorbents were obtained from Merck (Darmstadt, G.F.R.); silicone DC 200 was obtained from Applied Science Labs. (State College, Pa., U.S.A.). The test compounds were obtained from drug companies and their structures are reported in Table I.
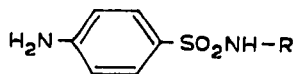
RESULTS AND DISCUSSION

The use of veronal acetate buffer at pH 7.4 as mobile phase gave suitable migrations of all of the compounds on silica gel G layers; the addition of acetone was not necessary. The R_M values are reported in Table I. On microcrystalline cellulose and Kieselguhr G layers, all of the compounds moved with the solvent front when the mobile phase was veronal acetate buffer alone or mixed with acetone. On polyamide layers, when the mobile phase was veronal acetate buffer alone, none of the compounds migrated from the starting line. However, the addition of acetone to the mobile phase gave increased migrations. Wang and Weinstein[11], by means of a polyamide layer and 95% ethanol–water (6:4) as mobile phase, obtained R_F values fairly close to those presently obtained in this work with 30% of acetone in the mobile phase. With 10% of acetone in the mobile phase, while eleven sulphonamides had R_M values between −0.62 and 0.59, that is, in a range that can be considered to be reliable, four compounds had R_M values between 1.16 and 1.59, which indicates too short a migration, and five sulphonamides did not move at all.

TABLE I

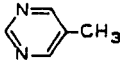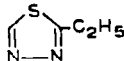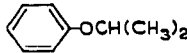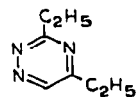### R_M VALUES OF SULPHONAMIDES ON SILICA GEL G AND POLYAMIDE

The R_M values on polyamide in the absence of acetone were calculated by means of the least-squares method using the experimental R_M values obtained with increasing acetone concentration in the mobile phase. For comparison the experimental R_M values at 10% acetone concentration are reported.

$$H_2N-\text{C}_6\text{H}_4-SO_2NH-R$$

No.	Compound	R	R_M values Silica gel G (0% acetone)	Polyamide 0% acetone (calcd.)	Polyamide 10% acetone
1	N'-Acetylsulphanilamide	COCH₃	−1.28	0.39	0.22
2	Sulphamerazine	(pyrimidinyl-CH₃)	−0.49	0.33	0.16
3	Sulphamethazine	(pyrimidinyl-(CH₃)₂)	−0.18	0.26	0.13
4	Sulphathiazole	(thiazolyl)	−0.71	0.74	0.59
5	Sulphanilamide	H	−0.98	−0.09	−0.24
6	Sulphamethoxypyridazine	(pyridazinyl-OCH₃)	−0.45	0.63	0.39
7	Sulphachloropyridazine	(pyridazinyl-Cl)	−0.89	1.85	1.59
8	Sulphamethoxydiazine	(pyrimidinyl-OCH₃)	−0.71	0.77	0.55
9	Sulphamethoxazole	(isoxazolyl-CH₃)	−1.02	1.49	1.19
10	Sulphadiazine	(pyrimidinyl)	−0.74	0.34	0.14
11	Sulphamethizole	(thiadiazolyl-CH₃)	−0.99	1.81	1.59
12	Sulphadimethoxine	(pyrimidinyl-(OCH₃)₂)	−1.02	1.86	—
13	Sulphisoxazole	(isoxazolyl-(CH₃)₂)	−1.07	1.87	—

TABLE I *(continued)*

No.	Compound	R	R_M values Silica gel G (0% acetone)	Polyamide 0% acetone (calcd.)	10% acetone
14	Sulphisomidine		−0.32	−0.58	−0.62
15	Isosulphamerazine		−0.37	0.75	0.54
16	Sulphaphenazole		−1.01	2.45	−
17	Sulphaethidole		−0.91	2.23	−
18	Sulphaproxyline		−0.73	1.68	−
19	Sulphapyridine		−0.20	0.40	0.17
20	Sulphasymazine		−0.77	1.45	1.16

In order to enhance the migration of all of the compounds, it was necessary to add acetone to the mobile phase. The experimental points in Fig. 1 show that with acetone concentrations of 10–50% it is possible to obtain migrations of most of the compounds. With 60% of acetone, each compound migrated separately. With 80% of acetone, all of the compounds tended to migrate with the solvent front. However, the plots in Fig. 1 show, for each compound, a linear region in the relationship between R_M values and acetone concentration in the mobile phase. More negative and/or lower R_M values indicate longer migrations of the compounds. The linear relationship between R_M values and composition of the mobile phase had been already shown for phenolic compounds[12], N-*n*-alkyltritylamines[13], penicillins[1], cephalosporins[2] and testosterone esters[14]. Boyce and Milborrow[13] pointed out that the R_M values in the linear region show maximum increments for each compound and among different compounds. Therefore, the fact that this linear relationship is also observed on polyamide layers is important because it makes it possible to calculate a theoretical R_M value in a standard system, where all compounds can be compared. The least-squares method was used to obtain the straight lines in Fig. 1. The theoretical R_M values with no acetone in the mobile phase were obtained by extrapolation from the linear region. Table I also gives the R_M values determined experimentally with 10% of acetone in the mobile phase. It can be seen that the extrapolation technique permitted
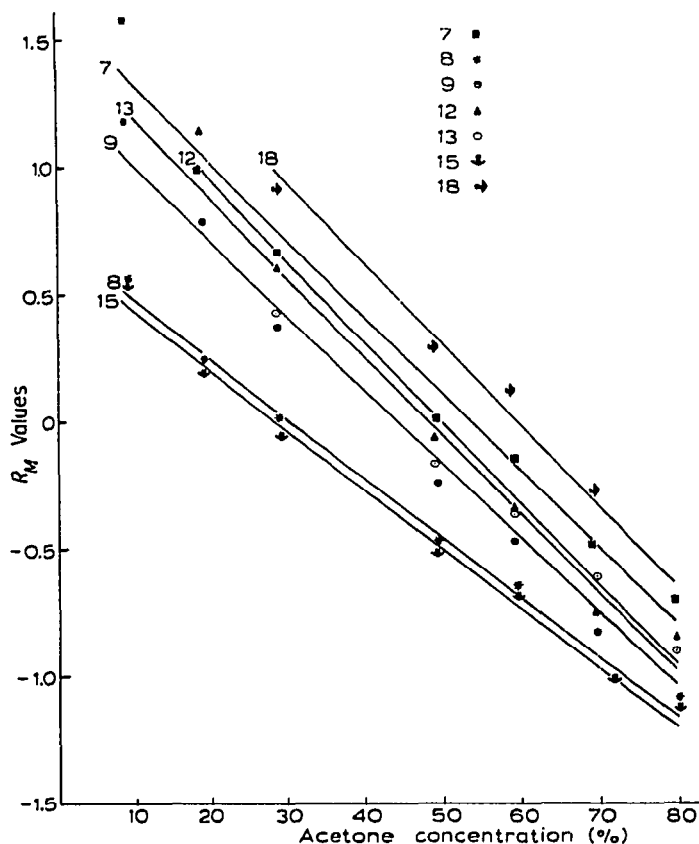
Fig. 1. Plots of R_M values for some sulphonamides against acetone concentration in the mobile phase with a polyamide layer as the stationary phase. The straight lines were calculated by means of the least-squares method. Compounds: 7, Sulphachloropyridazine; 8, Sulphamethoxydiazine; 9, Sulphamethoxazole; 12, Sulphadimethoxine; 13, Sulphisoxazole; 15, Isosulphamerazine; 18, Sulphaproxyline.

an R_M value with no acetone in the mobile phase to be obtained also for those compounds which did not move at lower acetone concentrations. The range of the calculated R_M values is much wider than that given by the experimental results. In this way, it is possible to obtain separate R_M values for compounds that otherwise could not be separated. The theoretical R_M values in the absence of acetone as well as those determined on silica gel G can show the influence of substituent groups and/or stationary phases on the chromatographic behaviour of sulphonamides. As the mobile phase was assumed to be the same in both systems, the different chromatographic behaviour of a given compound might be due to the nature of the stationary phases and therefore to the mechanism by which they separate organic compounds. Silica gel G and other inorganic materials function by virtue of a physicochemical attraction to the surface of the adsorbent; the balance between the hydrophilic and lipophilic character of substituents would play a major role. Polyamide acts by formation of a hydrogen bond between the amide linkage in the macromolecular polyamide and the compound; the steric effects of substituents seem to be very important

in determining chromatographic behaviour. It was shown that the steric effect of the methyl group in *o*-, *m*- and *p*-cresols is greater on polyamide than on silica gel G[11]. The difference between the R_F values of *o*-nitroaniline and *p*-nitroaniline is much larger on polyamide than on silica gel G[11].

In studies with sulphadiazine and its derivatives sulphamerazine, sulphamethazine, isosulphamerazine and sulphamethoxydiazine, it was found that on silica gel G the introduction of methoxy and methyl groups into sulphadiazine itself leads to a shorter migration, *i.e.*, a stronger interaction with the stationary phase. Sulphamethazine, with two methyl groups, showed the shortest migration (highest R_M values). In fact, the methoxy and methyl groups decrease the hydrophilic character of molecules[15]. However, it should be pointed out that a shorter migration could be due to a decrease in the interaction between water and the solute rather than to an increase in the interaction between the solute and silica gel G. On polyamide, while the strongest interaction was observed with sulphamethoxydiazine and isosulphamerazine, which are characterized by a methoxy and a methyl group, respectively, at C_5 in the pyrimidine ring, sulphamethazine had the lowest R_M value. In this instance, steric factors might be very important in determining the chromatographic behaviour. Moreover, the different position of the methyl group in sulphamerazine and isosulphamerazine influences the R_M value of the two compounds on polyamide more strongly than on silica gel G. Finally, it seems that the nature of the substituent at C_5 is less important on polyamide. In fact, while on silica gel G there is a difference between isosulphamerazine and sulphamethoxydiazine, on polyamide the two compounds behave very similarly. However, the influence of the methoxy group is not always clear. Replacement at the C_2 and C_6 positions of the two methyl groups of sulphisomidine with methoxy groups, as in sulphadimethoxine, resulted in a striking change in the R_M values on both polyamide and silica gel G. The bulkier methoxy groups could exert a steric effect that influenced the migration on polyamide; on silica gel G, the longer migration of sulphadimethoxine could be due to a relative hydrophilic effect of the methoxy groups. Replacement of the methoxy group of sulphamethoxypyridazine with a chlorine atom, as in sulphachloropyridazine, resulted in a longer and a shorter migration on silica gel G and polyamide, respectively. Replacement of a methyl group in sulphamethizole with an ethyl group, as in sulphaethidole, while not causing any evident change in the R_M value on silica gel G, resulted in a stronger interaction with the polyamide layer. The R_M value increased from 1.81 to 2.23 with a ΔR_M value of 0.42. It is interesting that almost the same difference occurs between sulphadiazine and isosulphamerazine ($\Delta R_M = 0.41$), which differ in the presence of a methyl group at the C_5 position in the pyrimidine ring. On the other hand, sulphamethazine, which differs from sulphamerazine by the presence of a second methyl group, did not show a similar increment in its R_M value. These effects further indicate the importance of the position of substituents in determining the chromatographic behaviour of sulphonamides on polyamide. It should be pointed out that the impregnation of the polyamide layer with silicone oil or other lipophilic substances did not influence the migration of the compounds, *i.e.*, the presence of silicone oil, squalane, etc., did not change the character of the interaction between the solute and the stationary phase. This is another important difference from the silica gel G layer, the impregnation of which changes the nature of the migration of sulphonamides.

CONCLUSION

The results obtained show the usefulness of polyamide layers for the chromatography of sulphonamides. Moreover, a comparison with the R_M values obtained on silica gel G indicate the different mechanisms by which the two stationary phases are likely to separate sulphonamides. Further work should be carried out on the relationship between the R_M values obtained on different stationary phases and biological activity. In particular, one could take into consideration the steric aspects of the interaction between the solute and the polyamide layer, which could provide a model for studying drug–receptor interactions involving steric influences.

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