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CHROMATOGRAPHIC AND ELECTROPHORETIC BEHAVIOUR OF SULPHONAMIDES ON THIN LAYERS OF ION EXCHANGERS

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SUMMARY

The chromatographic behaviour of fourteen sulphonamides and five N^4-derivatives has been widely investigated on strong and weak cation and anion exchangers with polystyrene, paraffin and cellulose matrices with aqueous and aqueous–organic eluents.

It has been demonstrated that the behaviour of sulphonamides is strongly correlated with the form in which such compounds are in solution and therefore with the protonic activity of the eluents. With aqueous organic eluents, it was possible to effect many interesting separations. The electrophoretic behaviour of sulphonamides on Bio-Rad AG 1-X4 (CH_3COO^-) and silica gel layers was dependent, as in thin-layer chromatography, on their acid–base characteristics.

INTRODUCTION

On the basis of the results achieved on thin layers of ion exchangers with purines and pyrimidines[1], aromatic amino acids[2] and primary aromatic amines[3], we considered it useful to extend our investigation to the sulphonamides, which have many similarities with these compounds. The sulphonamides have been studied on silica gel[4–10], alumina[7] and polyamide[7] layers, on columns of polystyrene-based cation exchangers with aqueous organic solvents[11] and, more recently, on papers impregnated with such exchangers with 1 *M* hydrochloric acid and dimethyl sulphoxide as eluents[12].

In this study we used polystyrene-, cellulose- and paraffin-based anion and cation exchangers. In the electrophoretic measurements, silica gel layers were also used.

EXPERIMENTAL

Solutions of most of the sulphonamides were prepared by dissolving the compounds in acetone. The N^4-derivatives of the sulphonamides were dissolved in water–acetone (2:1). The concentration of the solutions was 1–2 mg/ml.

Detection

The sulphonamides were detected with a 1% solution of *p*-dimethylamino-

benzaldehyde in 5% hydrochloric acid[5]. In the case of 5-sulphaminouracil and of the N^4-derivatives of the sulphonamides, the sprayed plates were heated for 5 min at 100°.

Preparation of the layers

Layers with a thickness of 300 μm were used. Dowex 50-X4 (H^+ and Na^+), Bio-Rad AG 1-X4 (CH_3COO^-) and Rexyn 102 (H^+ and Na^+) layers were prepared by mixing 2 g of the exchanger (200–400 mesh) and 6 g of microcrystalline cellulose in 40 ml of water. DEAE-cellulose (Cellex D) layers were prepared by mixing 6 g of the exchanger in 40 ml of water; a similar amount was employed for the microcrystalline cellulose layers. Alginic acid layers were obtained by mixing 3 g of the exchanger (prepared from SSDJ sodium alginate according to the procedure described in a previous paper[13]) with 6 g of microcrystalline cellulose in 40 ml of water.

The chromatographic measurements were carried out at 25°, using the Cryobox Desaga (Heidelberg, G.F.R.) chamber for thin-layer chromatography at constant temperature.

Electrophoretic measurements

The electrophoretic measurements were made with a Camag (Muttenz, Switzerland) apparatus for high-potential electrophoresis at 18°.

RESULTS AND DISCUSSION

Cation exchangers

Dowex 50-X4 (H^+). On this exchanger, when eluting with 1 and 2 *M* hydrochloric acid, the sulphonamides are strongly retained, with the exception of 5-sulphaminouracil. With hydrochloric acid solutions in water–ethanol mixtures, a notable decrease in the retention is observed. It should be noted, however, that the elution time gradually increases from 50 min in aqueous solution to 3 h in water–ethanol (1:4).

In Table I are listed the R_F values of fourteen sulphonamides and of sulphanilic acid on Dowex 50-X4 (H^+) eluting with 1 *M* hydrochloric acid solutions in water–ethanol mixtures at three different ratios. Sulphanilic acid is included as it may be present as a contaminant in some sulphonamides[14]. The results in Table I show that for most sulphonamides an increase in R_F values is observed as the proportion of ethanol in the aqueous solution is increased up to 50%. A small increase in the R_F values occurs or they remain constant with further increases in the proportion of ethanol. In the case of sulphallantoin, a decrease in the R_F value is observed. The different chromatographic behaviour of the sulphonamides when eluting with 1 *M* hydrochloric acid in water–ethanol mixtures and in aqueous solution may be attributed (a) to a decrease in the adsorption of the sulphonamides by the ion-exchanger matrix owing to the presence of ethanol inside the ion exchanger and (b) to a liquid–liquid partition effect due to a different distribution of the two components of the eluent between the solution and the ion exchanger at high proportions of ethanol[15]. Thus the behaviour of 5-sulphaminouracil and of sulphanilic acid, which exhibit decreasing R_F values at low proportions of ethanol, may be ascribed to their lower solubility in the aqueous–organic eluent rather than to a liquid–liquid partition effect.

Of the possible separations foreseeable on the basis of the R_F values, we ef-

TABLE I

R_F VALUES OF SULPHONAMIDES ON DOWEX 50-X4 (H^+) THIN LAYERS

Eluents: 1 *M* hydrochloric acid in aqueous ethanol; water–organic solvent mixtures.

No.	*Sulphonamide*	*1 M HCl in H_2O–EtOH*			*H_2O–EtOH*	*H_2O–CH_3CN*	*H_2O–DMSO*
		4:1	*1:1*	*3:7*	*1:4*	*1:4*	*1:4*
1	Sulphathiazole	0.13	0.31	0.34	0.02	0.04	0.36
2	Sulphaguanidine	0.02	0.03	0.03	0.00	0.00	0.03
3	Sulphamerazine	0.08	0.13	0.13	0.00	0.02	0.57
4	Sulphadiazine	0.14	0.22	0.25	0.02	0.05	0.63
5	Sulphamethazine	0.05	0.07	0.08	0.00	0.01	0.43
6	Sulphanilamide	0.28	0.37	0.37	0.02	0.04	0.29
7	Sulphanilic acid	0.82	0.75	0.72	0.61	0.41	0.98
8	Sulphabenzamide	0.06	0.28	0.37	0.15	0.61	0.88
9	Sulphacetamide	0.23	0.39	0.39	0.11	0.34	0.87
10	Sulphapyridine	0.01	0.02	0.03	0.00	0.00	0.20
11	Sulphisomidine	0.00	0.01	0.03	0.00	0.00	0.00
12	Sulphisoxazole	0.10	0.35	0.41	0.15	0.52	0.86
13	Sulphallantoin	0.27	0.35	0.32	0.01	0.05	0.27
14	2-Sulphanilamido-quinoxaline	0.03	0.17	0.23	0.08	0.18	0.78
15	5-Sulphaminouracil	0.62	0.46	0.34	0.85	0.70	0.98

fected the following: sulphanilamide, sulphathiazole, sulphapyridine, 5-sulphaminouracil and sulphanilic acid (4:1 water–ethanol); sulphabenzamide and sulphacetamide (4:1 water–ethanol); 2-sulphanilamido-quinoxaline, sulphallantoin and 5-sulphaminouracil (4:1 water–ethanol); sulphadiazine and sulphamethazine (3:7 water–ethanol); sulphapyridine and sulphadiazine (3:7 water–ethanol).

In Table I are also reported the R_F values obtained on Dowex 50-X4 (H^+) when eluting with water–ethanol, water–acetonitrile and water–dimethyl sulphoxide mixtures in a 1:4 ratio in the absence of hydrochloric acid. With water–ethanol (1:4), most compounds are strongly retained, with the exception of 5-sulphaminouracil and sulphanilic acid. Similar behaviour is observed with water–acetonitrile (1:4) although the retention of some sulphonamides is sharply decreased. With water–dimethyl sulphoxide mixtures, however, the sulphonamides generally show high R_F values, with the exception of sulphaguanidine and sulphisomidine, which remain virtually at the starting point. In this case, however, the elution time is very high (about 6 h) and elongated spots are obtained which render the separations of the different compounds very difficult. In fact, when eluting with water–dimethyl sulphoxide (3:7), it is not possible to separate sulphadiazine, sulphamerazine and sulphamethazine, although their R_F values are sufficiently different (see Table II).

The behaviour of the sulphonamides with aqueous organic eluents may be explained on the basis of the following relationship:

$$R\text{–}SO_3^- \; H^+ + B \leftrightharpoons R\text{–}SO_3^- \; BH^+ \qquad (1)$$

where B is the sulphonamide. This reaction is influenced in different ways by different types of organic solvent, depending on its acid–base characteristics. The more basic the solvent, the more reaction 1 is shifted towards the left. In fact, on changing from

TABLE II

R_F AND pK_a[11,12,17] VALUES FOR SOME SULPHONAMIDES ON DOWEX 50-X4 (H^+) THIN LAYERS

Sulphonamide	*H_2O–DMSO*			*H_2O–CH_3CN (1:4)*	*H_2O–EtOH (1:4)*	pK_{a_2}	pK_{a_3}
	2:3	*3:7*	*1:4*				
Sulphabenzamide	0.37	0.70	0.88	0.61	0.15	4.57	
Sulphacetamide	0.39	0.70	0.87	0.34	0.11	5.38	
Sulphadiazine	0.28	0.48	0.63	0.05	0.02	6.48	
Sulphamerazine	0.15	0.36	0.57	0.02	—	7.06	
Sulphamethazine	0.08	0.23	0.43	—	—	7.37	
Sulphanilamide	0.13	0.19	0.29	—	—	10.43	
Sulphathiazole	0.11	0.29	0.36	0.04	—	7.12	2.44*
Sulphapyridine	0.02	0.10	0.20	0.00	—	1.0	8.43
Sulphaguanidine	0.01	0.03	0.03	0.00	—	0.5	11.2

* pK_a of thiazole.

ethanol and acetonitrile to the more basic dimethyl sulphoxide[16], reaction 1 is shifted noticeably towards the left, with a consequent increase in the R_F values of most sulphonamides.

In order to explain the different retentions of sulphonamides by the exchanger in the case of a given eluent, the following facts must be borne in mind: (a) aromatic amines with pK_a values $\geqslant 2$ are strongly retained by the exchanger under the same elution conditions[3]; and (b) aminobenzenesulphonic acids with pK_a values between 2.48 and 3.75 have high R_F values on the same exchanger with the same eluents[2]. As the pK_{a_1} value of the sulphonamides is *ca.* 2, a negative charge, as in the case of aminobenzene sulphonic acids, should be present in the molecule of those sulphonamides whose retention is low. The presence in the molecule of a negative charge greatly decreases the affinity of the compounds towards the exchanger[2]. The possibility of assuming a negative charge is connected with the presence of an acidic group in the molecule; for this reason, these compounds may be considered to be similar to aromatic amino acids. On the basis of the pK_a values of the sulphonamides, most sulphonamides are in the anionic form at the pH of the eluent. In Table II are compared the R_F values obtained when eluting with aqueous organic solvents and the pK_{a_2} values found in the literature. The good agreement between the R_F and pK_{a_2} values should be noted, even if with water–dimethyl sulphoxide mixtures some exceptions are observed, such as sulphabenzamide and, only in the case of the 2:3 mixture, sulphanilamide. The peculiar behaviour of these compounds may be ascribed to liquid–liquid partition effects, as the behaviour of sulphabenzamide with water–ethanol and water–acetonitrile seems to demonstrate.

In the case of sulphathiazole, sulphapyridine and sulphaguanidine, which contain a group with more basic characteristics than the aromatic $-NH_2$ group, there must be a different explanation. The chromatographic behaviour of these compounds is noticeably affected by this basic group, as it may give rise to a further acid–base interaction with the sulphonic group of the ion exchanger. In the case of these three compounds, the R_F sequence is opposite to that of their pK_{a_3} values instead of that of the pK_{a_2} values.

Dowex 50-X4 (Na^+), sodium carboxymethylcellulose (CMCNa) and Rexyn 102 (Na^+). On CMCNa and Rexyn 102 (Na^+), the sulphonamides are retained only very slightly and high R_F values are generally obtained when eluting with water and with acetate buffer solutions. On Dowex 50-X4 (Na^+), however, when eluting with 0.5 *M* acetate buffer, the sulphonamides are strongly retained (R_F between 0.1 and 0.3) with the exception of 5-sulphaminouracil ($R_F = 0.80$) and of sulphanilic acid ($R_F = 0.95$). Such behaviour is correlated with the anionic (water as eluent) or neutral (acetate buffer as eluent) form of these compounds. The higher retention on Dowex 50-X4 (Na^+) than on CMCNa and Rexyn 102 (Na^+) when eluting with acetate buffer solutions may be ascribed to the stronger adsorption of the neutral form of the sulphonamides by the polystyrene matrix of the ion exchanger; such adsorption is very much less in the case of cellulose and paraffin matrices.

Alginic acid, Rexyn 102 (H^+). On these exchangers, the best results were achieved with 1 *M* acetic acid as eluent, as the chromatograms in Fig. 1 show. On alginic acid (Fig. 1a), in addition to many separation possibilities, very compact spots

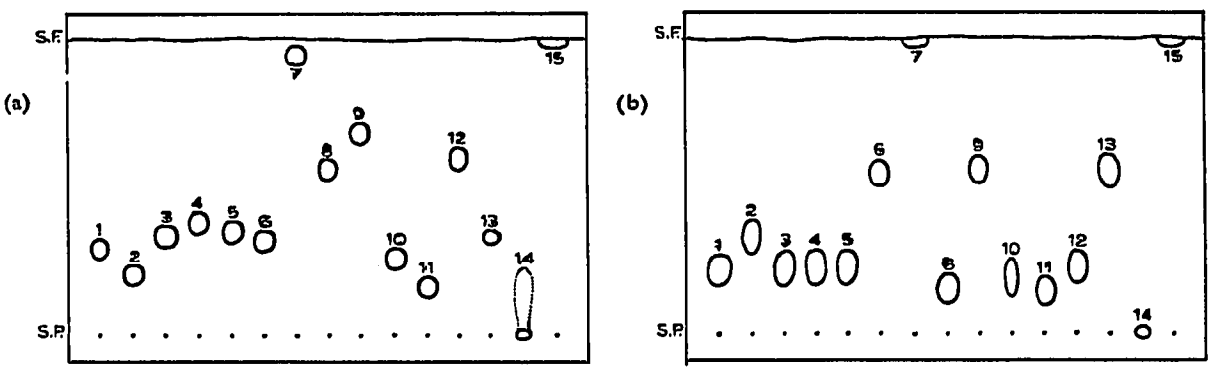

Fig. 1. Chromatograms of sulphonamides on thin layers of (a) alginic acid and (b) Rexyn 102 (H^+). Eluent: 1 *M* acetic acid. The numbers refer to the sulphonamides listed in Table I.

are obtained. From the comparison between the two chromatograms, many differences in the behaviour of the sulphonamides can be seen; such differences may be attributed to the different matrices of the two ion exchangers. In particular, sulphabenzamide and, to a lesser extent, sulphapyridine have a higher affinity towards the paraffin matrix than towards the cellulose matrix, while sulphallantoin, sulphanilamide and sulphaguanidine behave in the opposite manner.

On both exchangers, those separations which could be predicted on the basis of the R_F values have been effected.

Anion exchangers

Bio-Rad AG 1-X4 (CH_3COO^-). In Table III are listed the R_F values of the sulphonamides on Bio-Rad AG 1-X4 (CH_3COO^-) layers eluting with (a) 0.1 *M* acetate buffer, (b) 1 *M* acetic acid, (c) 2 *M* acetic acid and (d) 1 *M* acetic acid in water–ethanol (4:1). It should be noted that, as the pH of the eluent is decreased, higher R_F values are generally obtained, and that for a given acid concentration, a further increase in the R_F values is observed if ethanol is added to the eluent. An exception occurs, as

TABLE III

R_F VALUES OF SULPHONAMIDES ON AG 1-X4 (CH_3COO^-) THIN LAYERS

Eluents: (a) 0.1 *M* acetate buffer; (b) 1 *M* acetic acid; (c) 2 *M* acetic acid; (d) 1 *M* acetic acid in water–ethanol (4:1).

Sulphonamide	*Eluent*			
	a	*b*	*c*	*d*
Sulphathiazole	0.02	0.06	0.17	0.25
Sulphaguanidine	0.12	0.23	0.42	0.44
Sulphamerazine	0.09	0.20	0.37	0.47
Sulphadiazine	0.07	0.16	0.32	0.42
Sulphamethazine	0.11	0.26	0.45	0.52
Sulphanilamide	0.14	0.23	0.38	0.37
Sulphanilic acid	0.00	0.00	0.00	0.00
Sulphabenzamide	0.00	0.00	0.01	0.03
Sulphacetamide	0.05	0.12	0.23	0.27
Sulphapyridine	0.07	0.20	0.36	0.41
Sulphisomidine	0.14	0.48	0.75	0.67
Sulphisoxazole	0.00	0.02	0.06	0.13
Sulphallantoin	0.15	0.25	0.39	0.41
2-Sulphanilamidoquinoxaline	0.00	0.00	0.01	0.06
5-Sulphaminouracil	0.00	0.00	0.00	0.00

expected, in the case of sulphanilic acid and 5-sulphaminouracil, which remain at the starting point.

When eluting with 1 *M* acetic acid in aqueous solutions and with 2 *M* acetic acid, 5-sulphaminouracil gives rise, in addition to the spot at the starting point, to a second spot with a high R_F value (0.71 and 0.80 with 1 and 2 *M* acetic acid, respectively). Such a spot is associated with a hydrolysis product of 5-sulphaminouracil. The behaviour of the sulphonamides on this ion exchanger is correlated with the form of the compounds at the pH of the eluent. In 0.1 *M* acetate buffer, the sulphonamides are mostly in the neutral form and are strongly retained by the ion exchanger[1]. In 1 and 2 *M* acetic acid, the sulphonamides are mostly in the cationic form, which has a lower affinity towards the ion exchanger, as shown in the case of aromatic amines[3].

As regards the chromatographic behaviour of sulphonamides when eluting with 1 *M* acetic acid in water–ethanol mixtures, owing to the presence of ethanol a sharp decrease in the adsorption of the compounds by the ion-exchanger matrix is observed. The water–ethanol mixtures may be used, from an analytical point of view, both for the small amount of sulphonamide that may be employed and to give compactness of the spots more satisfactorily than with aqueous solutions. In order to illustrate the influence of acidity on the chromatographic behaviour of sulphonamides with aqueous organic eluents, in Fig. 2 are compared the chromatograms obtained when eluting with 1 *M* acetic acid in water–ethanol (1:1) and with water–ethanol (1:1), respectively. It may be noted that the presence of acetic acid is necessary in order to separate most sulphonamides, as the liquid–liquid partition effect is the determining factor only in the case of sulphaguanidine, sulphanilamide and sulphallantoin.

Cellex D (ClO_4^-), microcrystalline cellulose. In order to have a complete picture of the chromatographic behaviour of the sulphonamides, we considered it

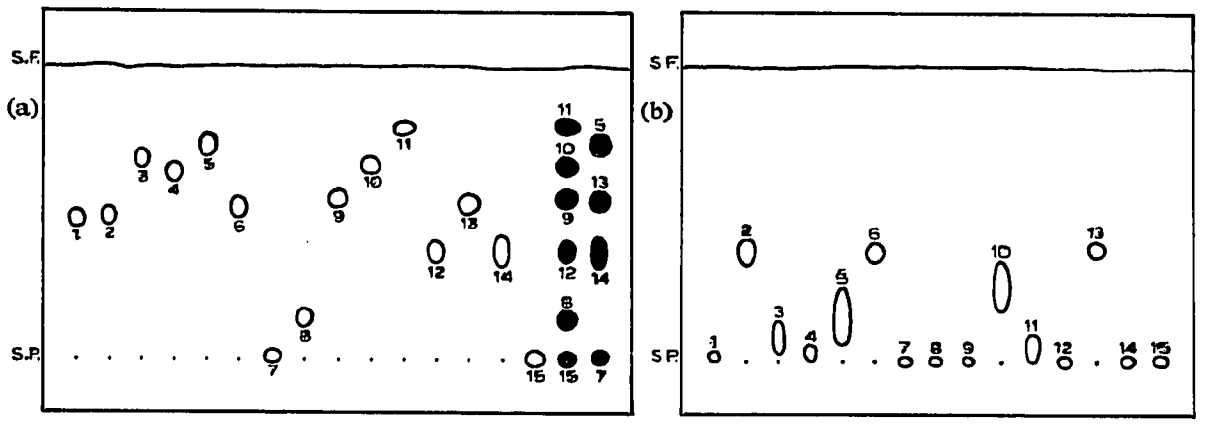

Fig. 2. Chromatograms of sulphonamides on AG 1-X4 (CH_3COO^-) thin layers. Eluents: (a) 1 *M* acetic acid in water–ethanol (1:1); (b) water–ethanol (1:1). The numbers refer to the sulphonamides listed in Table I.

useful to report the results for a cellulose-based anion exchanger and microcrystalline cellulose, which is a component of all the layers employed previously.

In Table IV there are reported the R_F values obtained when eluting with (a) water, (b) 0.01 *M* sodium perchlorate solution and (c) 0.1 *M* sodium perchlorate solution on microcrystalline cellulose and Cellex D. The remarkable differences observed on the two layers may be ascribed to the ion-exchange process on the Cellex D layers, as most sulphonamides are in the anionic form at the pH of the eluent.

TABLE IV

R_F VALUES OF SULPHONAMIDES ON MICROCRYSTALLINE CELLULOSE AND CELLEX D (ClO_4^-) THIN LAYERS

Eluents: (a) water; (b) 0.01 *M* sodium perchlorate solution; (c) 0.1 *M* sodium perchlorate solution.

Sulphonamide	*Microcrystalline cellulose*		*Cellex D (ClO_4^-)**		
	a	*c*	*a*	*b*	*c*
Sulphathiazole	0.73	0.70	0.02	0.10	0.42
Sulphaguanidine	0.73	0.70	0.62	0.55	0.60
Sulphamerazine	e.s.**	e.s.	0.04	0.20	0.71
Sulphadiazine	e.s.	e.s.	0.03	0.22	0.72
Sulphamethazine	0.84	0.83	0.03	0.20	0.68
Sulphanilamide	0.70	0.67	0.60	0.58	0.62
Sulphanilic acid	0.95	0.93	0.02	0.19	0.70
Sulphabenzamide	0.90	0.81	0.01	0.13	0.51
Sulphacetamide	0.95	0.83	0.02	0.25	0.79
Sulphapyridine	0.78	0.75	0.11	0.22	0.63
Sulphisomidine	0.87	0.85	0.01	0.14	0.53
Sulphisoxazole	0.93	0.77	0.01	0.15	0.56
Sulphallantoin	0.71	0.66	0.60	0.58	0.62
2-Sulphanilamidoquinoxaline	0.00	0.00	0.00	0.05	0.25
5-Sulphaminouracil	0.93	0.77	0.00	0.04	0.62

* Length of run 13 cm.

** e.s. = elongated spot.

The behaviour of sulphaguanidine, sulphanilamide and sulphallantoin is unusual on the Cellex D layers, as these compounds show high R_F values with all the eluents. Such behaviour may be attributed to the adsorption of these compounds, which are not in the anionic form (sulphanilamide is in the neutral form), by the cellulose matrix of the ion exchanger. Their behaviour is, in fact, similar to that of most sulphonamides on microcrystalline cellulose layers under the same elution conditions. It is interesting to note that the adsorption of sulphonamides is generally constant and increases only in some instances when changing from water to saline solutions. From an analytical point of view, some interesting separations may be effected on the Cellex D layers, as compact spots are obtained and the elution time is low (about 20 min).

N^4-derivatives of sulphonamides

These compounds (purchased from K & K, Plainview, N.J., U.S.A.) were studied in order to investigate the influence on the chromatographic behaviour of sulphonamides of the substitution of a hydrogen atom of the aromatic $-NH_2$ group with maleyl and phthalyl groups. The best results were obtained on Cellex D (ClO_4^-) layers, in terms of both the compactness of the spots and the difference in the R_F values which renders some separations possible. The R_F values obtained when eluting with 0.1 *M* sodium perchlorate solution are reported in Table V. The results in Table V show that the introduction of a maleyl or phthalyl group results in a sharp decrease in the R_F value of the substituted compound compared with the unsubstituted sulphonamide, but there are no differences between the maleyl and phthalyl derivatives of a given sulphonamide. In the case of maleylsulphathiazole, two spots are obtained owing to partial hydrolysis which leads to the formation of thiazole.

TABLE V

R_F VALUES OF SOME SULPHONAMIDES AND THEIR N^4-DERIVATIVES ON CELLEX D (ClO_4^-) THIN LAYERS

Length of run 13 cm. Eluent 0.1 *M* sodium perchlorate solution.

N^4-Derivative	R_F	*Sulphonamide*	R_F
Maleylsulphathiazole	0.27	Sulphathiazole	0.42
Phthalylsulphathiazole	0.28		
Maleylsulphanilamide	0.53	Sulphanilamide	0.62
Phthalylsulphanilamide	0.55		
Phthalylsulphacetamide	0.64	Sulphacetamide	0.79

It is not possible to employ polystyrene-based anion exchangers, for instance Bio-Rad AG 1-X4 (CH_3COO^-), because, owing to the high affinity of these compounds towards the ion-exchanger matrix, the N^4-derivatives remain virtually at the starting point when eluting with 1 *M* acetic acid in water or with 0.5 *M* acetate buffer in water–ethanol mixtures. In both cases, the N^4-derivatives undergo partial hydrolysis which leads to the formation of unsubstituted sulphonamides. On Dowex 50-X4 (H^+) layers with 1 *M* hydrochloric acid in aqueous solutions a behaviour similar to that observed on Bio-Rad AG 1-X4 (CH_3COO^-) with 1 *M* acetic acid as eluent is also ob-

served. In this second case, however, the hydrolysis of the N^4-derivatives occurs to a greater extent.

Electrophoretic measurements

As the results in Table VI show, the electrophoretic behaviour of sulphonamides is determined by their acid–base characteristics, at least for those compounds whose pK_a values are known (see Table II). In fact, as the basic strength of the compounds increases, an increase in the migration distance is observed. Such behaviour is similar on both Bio-Rad AG 1-X4 (CH_3COO^-) and silica gel layers. On mixing Bio-Rad AG 1-X4 (CH_3COO^-) with silica gel instead of microcrystalline cellulose, some advantages are observed in the electrophoretic measurements, because on such layers a smaller amount of sulphonamide can be used and longer migration distances are obtained.

TABLE VI

MIGRATION DISTANCES (mm) OF SULPHONAMIDES ON ANION EXCHANGERS AND SILICA GEL THIN LAYERS WITH 2 *M* ACETIC ACID AS ELECTROLYTE WITH MIGRATION TIMES OF 60 AND 90 min

Electric potential: 1100 V.

Sulphonamide	*Bio-Rad AG 1-X4 (CH_3COO^-)*	*Bio-Rad AG 1-X4 (CH_3COO^-) + silica gel D**		*Silica gel D***
	90 min	*60 min*	*90 min*	*60 min*
Sulphabenzamide	0	0	0	27
Sulphacetamide	8	12	21	35
Sulphadiazine	20	24	42	60
Sulphamerazine	26	29	51	71
Sulphamethazine	31	33	56	71
Sulphanilamide	30	38	62	104
Sulphathiazole	9	17	30	79
Sulphapyridine	23	32	52	89
Sulphaguanidine	32	39	68	125
Sulphanilic acid	− 4	− 5	− 8	−13
Sulphisomidine	53	63	116	127
Sulphisoxazole	2	3	5	31
Sulphallantoin	28	38	62	104
2-Sulphanilamidoquinoxaline	0	0	1	e.s.***
5-Sulphaminouracil	−13	−18	−26	n.d.§

* 2 g of AG 1-X4 (CH_3COO^-) + 12 g of silica gel D in 40 ml of water.
** 12 g of silica gel D in 40 ml of water.
*** e.s. = elongated spot.
§ n.d. = not determined.

It should be noted that on changing from microcrystalline cellulose to silica gel, no advantages are observed in thin-layer chromatography, because the silica gel causes a decrease in the compactness of the spots. On Bio-Rad AG 1-X4 (CH_3COO^-), mixed both with silica gel and microcrystalline cellulose, the effect of the electroosmotic flow is noticeably decreased[3] and therefore more reproducible results are obtained. On the basis of the migration distances, especially on Bio-Rad AG 1-X4 + silica gel layers, many separations are foreseeable.

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