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THIN-LAYER CHROMATOGRAPHY OF SULPHONAMIDES ON AMMONIUM MOLYBDOPHOSPHATE LAYERS

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SUMMARY

The use of ammonium molybdophosphate in the thin-layer chromatography of several sulphonamides has been investigated. The influence on the chromatographic behaviour of the concentration of the exchanger on the layer, temperature, acidity and salt concentration in the eluent and the type and concentration of organic solvent is discussed.

INTRODUCTION

Among the synthetic inorganic exchangers, ammonium molybdophosphate (AMP) is one of the more widely used for the separation of inorganic ions on columns[1–7], impregnated papers[8–11] and thin layers[12], as it exhibits a marked selectivity towards the alkali metal ions[1,2]. In contrast to zirconium phosphate, which has been employed on impregnated papers in the study of amino acids[13] and alkaloids[14], ammonium molybdophosphate has never, to our knowledge, been applied to organic compounds, notwithstanding its characteristics and the availability of a microcrystalline form (Bio-Rad Labs., Richmond, Calif., U.S.A.) which can be used in thin-layer chromatography (TLC).

We therefore investigated the use of AMP in the separation of organic compounds. This paper concerns sulphonamides, which have already been studied on polystyrene and cellulose-based ion exchangers[15] and by soap TLC[16], so that the different supports can be compared.

EXPERIMENTAL

Standard solutions of the sulphonamides (Carlo Erba, Milan, Italy, and K&K Labs., Plainview, N.Y., U.S.A.) were prepared by dissolving the compounds in acetone; sulphanilic acid was dissolved in water. The concentration of the solutions was 1–2 mg/ml and the amount of compound deposited on the layers was between 1 and 2 μg.

Detection

The sulphonamides were detected with a solution of 5% N,N-dimethyl-*p*-aminobenzaldehyde in ethanol–glacial acetic acid (5:1). The compounds appeared as orange spots on a yellow background; the detection was easier if the wet layers were sprayed.

Preparation of the layers

The layers (thickness 300 μm) were prepared with a Chemetron automatic apparatus by mixing the desired amount of AMP (from 0.5 to 8 g) in 50 ml of water. To the suspension, shaken with a magnetic stirrer, 2 g of calcium sulphate hemihydrate (first passed through a 200-mesh sieve) were added. After 10 min, the aqueous slurry of the sorbent with the above-mentioned binder was spread.

Unless otherwise stated, all measurements were carried out at 25° (using the Crycbox Desaga chamber for TLC) and the migration distance was 11 cm.

RESULTS AND DISCUSSION

Influence of AMP concentration

In order to illustrate the influence of the exchanger and the binder on the chromatographic behaviour of the sulphonamides, Table I lists the chromatographic characteristics of the compounds on layers of calcium sulphate hemihydrate alone and mixed with AMP in different ratios, eluting with water. The elution time, which is about 35 min on calcium sulphate hemihydrate, decreases with increase in the amount of AMP in the layer. On layers prepared with 8 g of AMP and 2 g of binder, for instance, the elution time is about 20 min.

In the presence of calcium sulphate hemihydrate alone, the compounds run almost with the solvent front, with the exception of sulphaquinoxaline, which remains at the application point owing to its low solubility in water. On the addition of a small amount of ammonium molybdophosphate, a substantial increase in the retention of the sulphonamides is observed, the only exception being sulphanilic acid, which runs with the solvent front and may therefore be separed from all of the other compounds. As the concentration of AMP is increased, a correspondlng decrease in all of the R_F values is observed; a limiting R_F value is not reached, however, even at high concentrates of exchanger in the layer.

As regards the compactness of the spots, on the two layers with lower AMP concentrations and particularly on that with 20% AMP, diffuse spots (which still have a round shape) are obtained. On layers with higher exchanger concentrations, more compact spots are obtained and the presence of a second spot, other than that which remains at the application point, can be seen with sulphaquinoxaline.

The affinity sequence of the sulphonamides changes as the exchanger concentration in the layer increases; the greatest differences are observed between the layers at lower AMP contents, as the R_F values of sulphabenzamide and sulphacetamide demonstrate.

The influence of substituent groups on the chromatographic behaviour of the sulphonamides can be deduced by comparison with the last three R_F values for sulphanilamide. The replacement of the amidic hydrogen atom by different groups results in a sharp increase in the affinity towards the stationary phase with the exception of

TABLE I

R_F VALUES OF SULPHONAMIDES AND SULPHANILIC ACID ON THIN LAYERS OF CALCIUM SULPHATE HEMIHYDRATE ALONE AND MIXED WITH INCREASING AMOUNTS OF AMP

Eluent: water.

Sulphonamide	$CaSO_4 \cdot \frac{1}{2}H_2O$	*AMP:$CaSO_4 \cdot \frac{1}{2}H_2O$ ratio*					pK_{a2} *(ref. 15)*
		*0.5:2**	*1:2*	*2:2*	*4:2*	*8:2*	
Sulphathiazole	0.96	0.39	0.23	0.16	0.10	0.05	7.12
Sulphaguanidine	0.97	0.62	0.44	0.35	0.20	0.14	0.5
Sulphamerazine	0.96	0.39	0.22	0.15	0.07	0.05	7.06
Sulphadiazine	0.97	0.47	0.29	0.22	0.11	0.07	6.48
Sulphamethazine	0.96	0.39	0.20	0.14	0.06	0.04	7.37
Sulphanilamide	0.97	0.84	0.68	0.66	0.43	0.33	10.43
Sulphanilic acid	0.98	0.98	0.97	0.97	0.88	0.82	—
Sulphabenzamide	0.97	0.65	0.55	0.51	0.33	0.23	4.57
Sulphacetamide	0.97	0.65	0.47	0.40	0.24	0.16	5.38
Sulphapyridine	0.97	0.43	0.28	0.20	0.09	0.05	1.0
Sulphisomidine	0.97	0.21	0.08	0.02	0.02	0.01	—
Sulphisoxazole	0.97	0.64	0.47	0.40	0.22	0.15	—
Sulphallantoin	0.97	0.84	0.68	0.66	0.44	0.34	—
Sulphaquinoxaline	0.00	0.00	0.00	0.00**	0.00**	0.00**	—
				0.18***	0.11***	0.10***	

* Diffuse spots are generally obtained.
** Main spot.
*** Secondary spot.

sulphallantoin, the R_F values of which are similar to those of sulphanilamide irrespective of the AMP concentration on the layer. It should be noted that the behaviour of these last two compounds is also similar on layers of organic ion exchangers[15] and in soap TLC[16].

The different retentions of the sulphonamides on AMP layers with elution with water can also be correlated with their pK_{a_2} values and, therefore, with their form in solution. As the behaviour of sulphanilic acid demonstrates, the presence in the molecule of a negative charge, greatly decreases the affinity of the compounds towards the exchanger. From this point of view, the behaviour of sulphabenzamide, sulphacetamide, sulphadiazine, sulphamerazine and sulphamethazine can be generalized: their sequence of affinities towards the exchanger is opposite to that of their pK_{a_2} values.

Sulphaguanidine and sulphapyridine do not seem to be affected by their pK_{a_2} values, which can be ascribed to their zwitterionic form owing to the presence in their molecules of a group with more basic characteristics ($pK_{a_3} = 11.2$ and 8.43, respectively) than the aromatic NH_2 group ($pK_{a_1} \approx 2$) of the sulphonamides. Therefore, the presence of a positive charge on the molecule causes an increase in the retention by the layer.

Influence of salt concentration and acidity of the eluent

The influence on the chromatographic behaviour of the sulphonamides of the type of eluent and the salt concentration in it is illustrated in Table II.

TABLE II

R_F VALUES OF SULPHONAMIDES AND SULPHANILIC ACID ON THIN LAYERS OF AMP + CALCIUM SULPHATE HEMIHYDRATE (4:1)

Sulphonamide	*Eluent*					
	NH_4NO_3			*$(NH_4)_2SO_4$ (1 M)*	*HNO_3 (0.5 M)*	*HNO_3 (0.5 M) + NH_4NO_3 (1 M)*
	1 M	*2 M*	*4 M*			
Sulphathiazole	0.10	0.18	0.28	0.08	0.02	0.11
Sulphaguanidine	0.29	0.44	0.53	0.31	0.03	0.20
Sulphamerazine	0.11	0.16	0.22	0.07	0.00	0.06
Sulphadiazine	0.12	0.20	0.25	0.09	0.01	0.09
Sulphamethazine	0.09	0.14	0.19	0.03	0.00	0.05
Sulphanilamide	0.45	0.61	0.64	0.43	0.20	0.39
Sulphanilic acid	0.76	0.86	0.85	0.81	0.83	0.74
Sulphabenzamide	0.22	0.32	0.36	0.11	0.09	0.16
Sulphacetamide	0.19	0.28	0.27	0.17	0.05	0.14
Sulphapyridine	0.12	0.21	0.27	0.10	0.00	0.05
Sulphisomidine	0.02	0.06	0.09	0.02	0.00	0.02
Sulphisoxazole	0.16	0.29	0.31	0.12	0.04	0.12
Sulphallantoin	0.43	0.60	0.65	0.44	0.19	0.40
Sulphaquinoxaline	0.00* 0.10**	0.00* 0.11**	0.00* 0.11**	0.00* 0.10**	0.00	0.05
1st front	0.76	0.85	0.85	0.82	0.82	0.75

* Main spot.
** Secondary spot.

The replacement of water with 1 *M* ammonium nitrate solution results in an increase in the R_F values, except for those of sulphaquinoxaline and sulphanilic acid. The appearance of a double front on the layer is observed. The behaviour of sulphaquinoxaline agrees with its low solubility in both water and salt solutions, as the constancy of the R_F values of the two spots on increasing the ammonium nitrate concentration and on changing the type of salt shows.

Sulphanilic acid runs with the first solvent front and therefore its behaviour is correlated with the R_F change of this front on changing the type of eluent the and salt concentration in it.

As the ammonium nitrate concentration in the eluent is increased, the sulphonamides exhibit increasing R_F values, even if for some of them (such as sulphacetamide and sulphisoxazole) a limiting value is reached for salt concentrations higher than 2 *M*.

The increase in the R_F values is greater with sulphapyridine and sulphaguanidine, probably owing to the presence in their molecules of a positive charge. The behaviour of these two compounds suggests the presence of a ion-exchange process in the retention mechanism. Such a process, however, must be excluded for all of the other sulphonamides which are not in the cationic form at the pH of the eluent.

The lower retention of the sulphonamides with respect to elution with water cannot be explained even on the basis of a probable decrease in the proportion of the anionic form present at higher acidities of the ammonium nitrate solutions (pH = 4.8), as a decrease in the R_F values should be observed. It follows, therefore, that the

most probable mechanism of the retention of sulphonamides is based on an adsorption process. The decrease in the R_F values on elution with ammonium sulphate instead of ammonium nitrate at the same ammonium ion concentration and with 2 *M* potassium nitrate solution supports the presence of an adsorption process.

On eluting with 0.5 *M* nitric acid, a sharp decrease in the R_F values in comparison with elution with water or salt solutions is observed. Only sulphanilic acid moves with the first solvent front. As in 0.5 *M* nitric acid all the sulphonamides are in the cationic form, a direct dependence of the affinity towards the exchanger on the form of the sulphonamides is supported.

On adding ammonium nitrate to the eluent, a sharp decrease in retention is observed even if the R_F values of the different compounds are lower than those obtained on eluting with 1 *M* ammonium nitrate solution alone. It should be noted that sulphaquinoxaline gives rise to only one spot owing to its higher solubility in strong acids than in water.

The retention of the sulphonamides with strongly acidic eluents is probably correlated with an ion-exchange process, even if the simultaneous occurrence of an adsorption process cannot be excluded.

Influence of temperature

In order to investigate the performance of AMP layers from an analytical standpoint, we carried out tests at temperatures between 25° and 45°. Fig. 1 shows the changes in R_F values for some sulphonamides and sulphanilic acid on elution with water. It can be seen that the R_F values increase with increasing temperature. Such behaviour agrees with the relationship between R_M and $1/T$ found by Lederer[17].

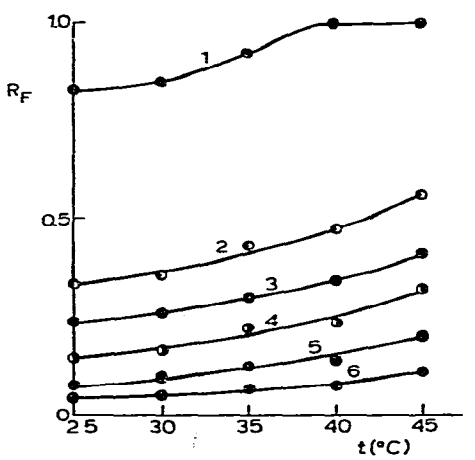

Fig. 1. Temperature dependence of R_F values on AMP + $CaSO_4 \cdot \frac{1}{2}H_2O$ (4:1) thin layers. 1, Sulphanilic acid; 2, sulphanilamide; 3, sulphabenzamide; 4, sulphaguanidine; 5, sulphadiazine; 6, sulphamethazine.

In addition to the increase in R_F values an improvement in the resolution of the compounds is also observed at higher temperatures.

From an analytical standpoint, elution at 45° can be used for the separation of eight compounds (sulphisomidine, sulphamethazine, sulphamerazine, sulphadiazine,

sulphaguanidine, sulphabenzamide, sulphanilamide and sulphanilic acid) the R_F values of which increase in the above-mentioned order. The elution time must be not less than 90 min.

Aqueous–organic eluents

In order to examine the influence of organic solvents on the chromatographic behaviour of the sulphonamides, we employed several aqueous–organic mixtures with the same ratio of water to organic solvent (1:1, v/v).

As the presence of the organic solvent considerably affects the chromatographic characteristics of the compounds in comparison with elution with aqueous solutions, a study with water–methanol mixtures was also carried out on layers with different AMP concentrations.

The main differences in comparison with elution with water are (1) the elution time (about 1.5 h), (2) the compactness of the spots even on layers with the lowest concentration of exchanger, (3) the R_F increase for all of the sulphonamides except sulphanilic acid, whose R_F value decreases, and (4) the affinity sequence.

As the data for elution with water–methanol mixtures show (Table III), the three sulphonamides, that differ only in the presence of a methyl group in the molecule (sulphadiazine, sulphamerazine and sulphamethazine) can be differentiated.

TABLE III

R_F VALUES OF SULPHONAMIDES AND SULPHANILIC ACID ON THIN LAYERS OF AMP + CALCIUM SULPHATE HEMIHYDRATE IN RATIOS OF (A) 0.5:2, (B) 2:2, (C) 4:2 AND (D) 8:2

Eluents: aqueous–organic solvents (1:1, v/v).

Sulphonamide	*Water–methanol*				*Water–acetonitrile (D)*	*Water–butyric acid (D)*
	(A)	*(B)*	*(C)*	*(D)*		
Sulphathiazole	0.80	0.28	0.18	0.14	0.22	0.10
Sulphaguanidine	0.88	0.39	0.28	0.21	0.29	0.16
Sulphamerazine	0.67	0.19	0.12	0.09	0.20	0.12
Sulphadiazine	0.78	0.28	0.17	0.13	0.24	0.20
Sulphamethazine	0.67	0.15	0.08	0.05	0.20	0.13
Sulphanilamide	0.94	0.70	0.55	0.46	0.45	0.57
Sulphanilic acid	0.95	0.92	0.83	0.82	0.92	0.63
Sulphabenzamide	0.94	0.61	0.44	0.40	0.36	0.84
Sulphacetamide	0.87	0.51	0.35	0.30	0.40	0.54
Sulphapyridine	0.64	0.20	0.11	0.07	0.15	0.08
Sulphisomidine	0.17	0.01	0.01	0.00	0.02	0.00
Sulphisoxazole	0.91	0.61	0.41	0.35	0.38	0.74
Sulphallantoin	0.93	0.70	0.56	0.47	0.46	0.61
Sulphaquinoxaline	0.25	0.24	0.19	0.15	0.17	0.66

On replacing methanol with ethanol, no major differences are observed in the behaviour of the sulphonamides; with acetonitrile a sharp increase in the R_F values is observed for most compounds, with exception of sulphanilamide, sulphabenzamide and sulphallantoin.

The use of more polar solvents, such as dimethylformamide and dimethyl

sulphoxide, must be avoided because of difficulties in detection and the occurrence of elongated spots which are formed with most compounds. Such an occurence is also observed on eluting with ethylene glycol.

With organic acids as eluents, the best results were achieved with water–butyric acid; with acids of lower molecular weight (*e.g.*, acetic acid) most compounds remain at the application point and the others give rise to elongated spots.

Considering the R_F values obtained with water–butyric acid as the eluent, the sequence of the affinities towards the exchanger is very different from those obtained with the other eluemts. Many sulphonamides, such as sulphabenzamide, sulphisoxazole and sulphaquinoxaline, exhibit higher R_F values than sulphanilic acid, while almost all of the others are more retained than with the above-mentioned aqueous–organic eluents. Such behaviour can be used for analytical purposes as compact spots are achieved. The elution time is about 2.5 h .

Influence of organic solvent concentration

Fig. 2 shows the R_F values of sulphanilic acid (curve 1) and some representative sulphonamides (curves 2–6) with increasing concentrations of methanol in the eluent. The trend with sulphanilic acid is correlated with its low solubility in solutions with a high methanol content. The sulphonamides exhibit constant R_F values at methanol concentrations less than 30%, a sharp increase at concentrations between 30 and 60% and a further constancy at higher concentrations.

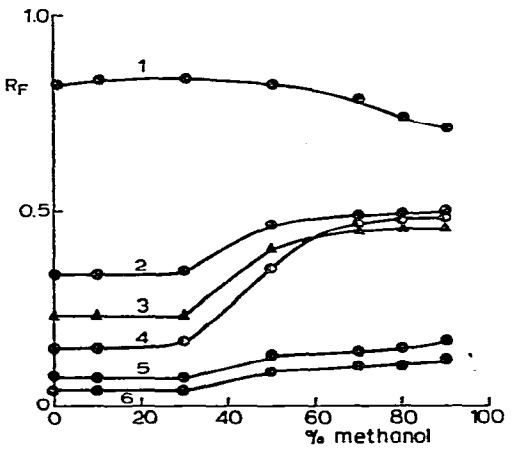

Fig. 2. R_F values of sulphonamides on AMP + $CaSO_4 \cdot \frac{1}{2}H_2O$ (4:1) thin layers *versus* methanol content in the eluent. 1, Sulphanilic acid; 2, sulphanilamide; 3, sulphabenzamide; 4, sulphisoxazole; 5, sulphadiazine; 6, sulphamerazine.

In contrast to the results observed on organic ion exchangers[15] and silanized silica gel impregnated with triethanolamine dodecylbenzenesulphonate[16], on layers of AMP levelling of the R_F values is not reached for methanol contents above 60%.

The difference in the R_F values between the sulphonamides in curves 2, 3 and 4 and those in curves 5 and 6 is more marked at high methanol concentrations than when water is used.

Analytical applications

Many separations can be carried out on AMP layers. Other than the above-mentioned separations on eluting with water at 45°, those in Table IV were effected at 25° on eluting with aqueous–organic mixtures. It should be noted that the composition of the layers has an influence as some separations can be effected only at those ratios of exchanger to calcium sulphate hemihydrate reported in Table IV.

TABLE IV

SEPARATIONS OBTAINED ON THIN LAYERS OF AMP + CALCIUM SULPHATE HEMIHYDRATE

Mixture	*AMP to $CaSO_4 \cdot \frac{1}{2}H_2O$ ratio*	*Eluent*	*R_F value*
Sulphathiazole	8:2	Water–butyric acid	0.10
Sulphacetamide		(1:1)	0.54
Sulphisoxazole			0.74
Sulphabenzamide			0.84
Sulphisomidine	8:2	Water–butyric acid	0.00
Sulphaquinoxaline		(1:1)	0.66
Sulphamethazine	4:2	Water–methanol*	0.10
Sulphamerazine		(1:1)	0.15
Sulphadiazine			0.21
Sulphisomidine	2:2	Water–methanol	0.01
Sulphamethazine		(1:1)	0.15
Sulphadiazine			0.28
Sulphaguanidine			0.39
Sulphanilamide			0.70
Sulphanilic acid			0.92

* Migration distance 13.5 cm. Elution time 3 h.

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