CHROM. 4852

Thin-layer chromatography of inorganic sulphur compounds

Procedures have been described for the separation of inorganic sulphur compounds by paper[1–5] and ion-exchange[6–8] chromatography, but few systems using thin-layer chromatography have been described[9,10]. Owing to the high reactivity of thiosulphate and polythionates in the mixtures encountered in metabolic studies of such compounds[1,7,11], a method for the rapid analysis of these mixtures was desirable. The procedures described below enable separations of sulphate, thiosulphate, trithionate, tetrathionate and thiocyanate to be made.

Experimental

Stationary phases were sheets of Gelman instant thin-layer chromatography (ITLC) media, types SA and SG (20 × 20 cm). Chromatograms were run in Gelman ITLC chambers at 22–25°. Marker compounds (usually 10 μg in 10 μl water) were applied 3 cm from the edge of the sheets and the solvent allowed to run 13 cm beyond the origin line. Sources of marker compounds were as follows: AnalaR grade KSCN and $K_2S_2O_3$ from the British Drug Houses; ^{35}S-labelled sulphate and thiosulphate from The Radiochemical Centre (Amersham, Great Britain); ^{35}S-labelled thiocyanate was synthesised by the cyanolysis of labelled tetrathionate; and potassium tetrathionate[12] and trithionate[13] were synthesised. Solvents were AnalaR or best commercial grades and were not further purified. Sulphur compounds were detected by spraying dried chromatograms with 8% (w/v) silver nitrate in acetone containing 10% (v/v) water, and by radioautography and liquid scintillation counting when ^{35}S-labelled materials were used.

Results and discussion

A number of solvents, including several suitable for paper chromatography, were generally unsatisfactory for TLC separations in this study (Table I). Solvent S_2 is very useful for paper chromatography[2], and did separate the four compounds tested. The rather large spots produced did, however, decrease the efficiency of sepa-

TABLE I

TLC OF THIONATES AND THIOCYANATE ON SA MEDIUM USING MIXED SOLVENTS

Solvents used were: S_1 1-butanol–acetone–water (40:40:30)
S_2 1-butanol–pyridine–acetic acid–water (30:20:6:24)
S_3 1-butanol–pyridine–acetic acid–water (90:3:1:6)
S_4 1-propanol–pyridine–water (50:35:50)
S_5 ethanol–pyridine–water (80:10:5)
S_6 ethanol–pyridine–water (60:30:10)
S_7 2-heptanol–methanol–water (85:10:5)

Compound	R_F						
	S_1	S_2	S_3	S_4	S_5	S_6	S_7
Thiosulphate	0.68	0.50	0.02	0.84	0.45	0.54	0.05
Trithionate	0.76	0.61	0.26	0.86	0.72	0.74	0.05
Tetrathionate	0.79	0.67	0.39	0.86	0.78	0.76	0.05
Thiocyanate	0.84	0.78	0.62	0.82	0.76	0.75	0.22

TABLE II

TLC OF INORGANIC SULPHUR COMPOUNDS ON SA MEDIUM USING ALCOHOLS AS SOLVENTS

Solvents used were: S_8 methanol
S_9 99% ethanol
S_{10} 5% (v/v) water in 1-propanol
S_{11} 5% (v/v) water in 1-butanol
S_{12} 5% (v/v) water in 1-pentanol
S_{13} 5% (v/v) water in 1-hexanol
S_{14} 1-octanol saturated with water
S_{15} 1-propanol–methanol (1:1)
S_{16} 5% (v/v) water in 2-butanol

Compound	R_F								
	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}
Sulphate	0[a]	0	0	0	—	—	—	0[a]	—
Thiosulphate	0.78	0.25	0.06	0.02	0.10	0	0	0.49	—
Trithionate	0.84	0.73	0.54	0.35	0.09	0.03	0.03	0.73	0.27
Tetrathionate	0.84	0.80	0.63	0.46	0.19	0.10	0.04	0.77	0.37
Thiocyanate	0.83	0.79	0.71	0.64	0.47	0.38	0.36	0.77	0.60
Approx. time for standard run (min)	45	85	140	180	300	390	520	90	240

[a] See text.

ration of thiocyanate and the polythionates from each other. Thiocyanate was separated effectively from all the other compounds only by solvent S_7.

In contrast, all four compounds and sulphate could be separated from each other using pure alcohols or alcohols containing small quantities of water (Table II). No single solvent effected perfect separation of all five compounds, but 5% (v/v) water in 1-butanol (S_{11}) gave complete separation of thiosulphate, trithionate, tetrathionate and thiocyanate, while methanol (S_8) or methanol–propanol (S_{15}) completely resolved sulphate from the other compounds. Solvent S_{15} allowed isolation of sulphate, thiosulphate and thiocyanate or polythionates from each other. Resolution of a mixture of all five compounds can thus be achieved by two-dimensional chromatography in solvents S_{15} and S_{11}. The behaviour of sulphate in solvents S_{15} and S_8 indicated S_{15} to be preferable. Sulphate showed no significant movement in ethanol or longer-chain alcohols, but tended to streak from the origin in solvents S_{15} and S_8. This streaking was more pronounced with S_8 and the distance streaked increased with the amount of sulphate on the thin layer. When 30 μg of sodium sulphate was applied, 80% remained within 2 cm of the origin, but the remainder streaked a further 7 cm in S_8. With methanol–propanol (S_{15}), 80–85% of the sulphate remained within 2 cm of the origin, most not migrating at all, and none moved further than 3.5 cm from the origin. The "tail" of the sulphate thus remained 1.5–2 cm behind the rear of a 20 μg spot of potassium thiosulphate.

Pure 1-propanol was an unsatisfactory solvent, as sulphate and thiosulphate remained at the origin, while trithionate and tetrathionate gave spots of R_F 0.36 and 0.54 but streaked from the origin.

Gelman ITLC SG medium was not generally suitable for these separations, although with solvent S_{14} thiocyanate (R_F 0.78) was separated from all the other compounds, which did not migrate.

In conclusion, chromatography on Gelman ITLC SA media using 5% water in 1-butanol and a 1:1 mixture (v/v) of methanol–1-propanol effects complete separation of sulphate, thiosulphate, trithionate, tetrathionate and thiocyanate in not more than 5 h. This technique is of use in analysing mixtures such as are encountered during bacterial sulphur metabolism[14,15] or during reaction of thionates with cyanide[16].

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First received May 12th, 1970; revised manuscript received June 4th, 1970