The Analysis of Inorganic Compounds by Paper Chromatography. Part VIII. The Separation of the Thionic Acids by a New Paper-chromatographic Technique.*

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The effect of a phase boundary on the separation of the thionic acids by use of an organic solvent containing potassium acetate is discussed with particular reference to the problem of double zoning and the formation of two mobile phase-regions on the chromatogram. A new technique is described which eliminates this double zoning, whereby the substances to be separated are placed on the paper after the phase boundary has passed the starting line. A solvent system is suggested for the complete separation of the potassium salts of the thionic acids by means of this technique.

THE qualitative separation of thionic acids has already been reported (Pollard, *Brit. Med. Bull.*, 1954, **10**, 191) by use of the solvent system *iso*propanol–acetone–aqueous potassium acetate and the normal downward technique, but some difficulties occurred owing to the formation of double zones for tri- and tetra-thionates when spotted as their potassium salts.

Later work has shown the existence of two phase-regions on the chromatogram, region I extending from the solvent front to an R_F value of *ca.* 0·5, and region II continuing to the solvent feed. The leading phase was acid and free from potassium ions, while the rear phase was alkaline to methyl-orange and contained potassium ions as detected by the lead–cobalt nitrate–sodium nitrite test (Pollard, McOmie, and Stevens, *J.*, 1951, 771).

Both tri- and tetra-thionate gave one zone in each region, no potassium ions being associated with the zones in region I; the latter zones must therefore contain free acids. Potassium penta- and hexa-thionate only appeared on the chromatogram as free acids in region I, while potassium thiosulphate occurred in region II.

Whenever the potassium thionates were chromatographed with the solvent corresponding to region I, double zoning occurred to a varying degree, depending on the thionate species. This was confirmed by running chromatograms in the following ways: (*a*) placing the thionates a long distance from the solvent feed, where region I is large, and ending the run before they were overtaken by region II; (*b*) eluting the thionates with a solvent in which acetic acid replaced potassium acetate, *i.e.*, to simulate the assumed conditions of region I; and (*c*) eluting the thionates with the leading phase corresponding to region I. The latter was prepared by a frontal analysis of the original solvent on a cellulose column.

The zones at lower R_F for each thionate were very well defined and were found to be associated with potassium ions, while no cations could be detected corresponding to the more diffuse zones at high values of R_F. The "acid" zone for thiosulphate and the

* Part VII, preceding paper.

potassium salt zone for pentathionate were of low concentration, and *vice versa*, while each of the double zones for tri- and tetra-thionate appeared roughly equal in concentration.

From these results it appears that, in the leading phase, the free thionic acids are formed by hydrolysis of the salts (hydrogen ions being produced from the acetic acid present) and are removed preferentially from the starting line. The amount of thionate converted into the acid will depend on the strength of the particular thionic acid and on its solubility in the solvent corresponding to region I.

When the moving phase-boundary overtakes the potassium salt zones, all conversion into the free acid is prevented. The position of the phase boundary relative to the solvent front can be quoted without referring to the starting line by measuring the R_P value, where

$$R_P = \frac{\text{distance of phase boundary from solvent feed}}{\text{distance of solvent front from solvent feed}}$$

Such a ratio should be used for defining the position of any fronts or zones when the substance causing the zone is a component of the solvent system. The R_P value for the above solvent system was found to be constant, independent of the length of run, provided the tank was fully equilibrated.

To avoid double zoning due to the above causes, a technique was developed whereby the filter-paper strip is irrigated with solvent until the moving phase-boundary is well past the starting line, whereupon the solutes are placed on the wet paper. It is proposed to call this technique "rear-phase chromatography;" the time of irrigation of the paper before addition of solutes is the pre-elution period, and the time of irrigation after the addition is the chromatographing period.

In this technique, R_F values for the ions cannot be given since the solvent front passes the end of the paper strip. Instead, the absolute distances moved by the ions are quoted in cm. The higher thionates move at a speed approaching that of the solvent front; these species will overtake the phase boundary if the pre-elution period is too short, and they will then produce zones in region I. Conditions for retention of the higher thionates within region II are given in the Experimental section. The distance of starting line from solvent feed is still important, a decrease in this distance being equivalent to a large increase in pre-elution period.

The conditions of rear-phase chromatography are the same as those on a cellulose column washed with solvent to effect equilibration before separation.

Experimental

Apparatus.—For the preliminary work, and the investigation of the formation of the phase boundary, the downward technique was used in conjunction with acid-washed Whatman No. 1 paper (Pollard and Banister, *Analyt. Chim. Acta*, in the press). The apparatus for rear-phase chromatography consists of a modification of the usual frame for supporting chromatograms, whereby two horizontal glass bars, about 1″ apart, on the same side of, and parallel to, the solvent trough, and raised about 1″ above the horizontal trough supports, replace the glass bar on which the chromatogram would normally rest.

The chromatographic strip is inserted in the solvent mixture (the end in the solvent being weighed down with a glass bar), so that the strip rests over the two glass bars mentioned, with a marked line on the strip in a position between them. This part of the strip is very close to the tank lid, which is provided with a slit ($\frac{1}{8}$″ × 6″) directly above the marked line. The slit remains covered with a piece of glass during the run, but is uncovered in order to admit the solutes to be separated.

Since the solvent flows off the bottom of the chromatogram in this technique, a filter-paper pad is clipped to the bottom (cf. Westman, Scott, and Pedley, *Chemistry in Canada*, 1952, **4**, 189). Acid-washed paper was unnecessary in rear-phase chromatography, since presumably much of the impurity is washed down the paper before the solutes are chromatographed.

Operating Technique.—The filter-paper strip is marked out with two lines parallel to the top of the strip, the first, 2 cm. from the top where an upward fold is made, the second, a distance d from this line, where d depends on the dimensions of the apparatus. In two apparatus used, d was 5 cm. and 7 cm. severally. When the paper strip is placed with the top end in the trough

as described above, the second line should be about midway between the two parallel bars. The smaller the value of d, the less pre-elution period is necessary.

In order to obtain a reproducible R_F value, about 10 hours' equilibration of the tank with the solvent mixture in the trough is found necessary before the paper is eluted. If only short equilibration times are used, the R_F value is greater than with true equilibration owing to the more rapid evaporation of region I.

After equilibration, the filter-paper strip is placed in the tank as described above, and is eluted for a length of time sufficient for the phase boundary to have moved the required distance past the starting line.

To mark the position of the phase boundary during the run, an indicator was added to the solvent system. Two indicators, bromocresol-purple and phenol-red, had sufficiently high R_F values and convenient pH ranges (5·2—6·8 and 6·8—8·4, respectively) for this purpose.

The prepared solutions of the solutes are then " spotted " on the wet paper at the marked starting line through the slit in the tank lid by means of glass capillary tubes. The slit is then re-covered, and the chromatogram eluted for a certain length of time. During the elution, colourless spots due to the water from the solute solutions are visible against the indicator-coloured background, moving with a velocity similar to that of the solvent front and hence overtaking the phase boundary after a while.

Samples.—Potassium tri-, tetra-, and penta-thionates were prepared by the methods of Goehring *et al.* (Stamm, Goehring, and Feldmann, *Z. anorg. Chem.*, 1942, **250**, 226; Goehring and Feldman, *ibid.*, 1948, **257**, 223). Potassium hexathionate was prepared by Weitz and Achterberg's method (*Ber.*, 1928, **61**, 399). The samples of potassium tri-, tetra-, and penta-thionate were *ca.* 97% pure, and the potassium hexathionate sample was about 90%, the only impurity being potassium chloride. Chromatographic analysis has shown that these samples do not contain detectable amounts of thionates other than the desired preparation. B.D.H. potassium thiosulphate monohydrate was also used.

0·1N-Solutions of each substance were freshly prepared, also a comprehensive mixture, 0·1N with respect to each component. Precipitated sulphur appeared in the mixture after a short time, owing to the catalysing action of thiosulphate on the decomposition of thionates in aqueous solution. Large quantities of penta- and tetra-thionate were detected in the hexa-thionate solution after about 12 hr., but the penta- and tetra-thionate solutions remained without appreciable decomposition for about 3 days (cf. Goehring, Helbing, and Appel, *Z. anorg. Chem.*, 1947, **254**, 189).

Owing to the paper's being wet when the solutes are spotted, the amount of solution to use cannot be gauged by the size of the spot produced. For qualitative purposes, the solutes were drawn into melting-point tubes (approx. diameter 1 mm.), and the tubes held in contact with the pre-eluted paper for about 3 sec.

Development.—The dried chromatograms are sprayed with 0·5N-silver nitrate solution, and warmed, whereupon the unstable silver salts of all these acids decompose, giving a brown stain of silver sulphide and sulphur. In order to keep the chromatograms, they are then washed in concentrated thiosulphate solution and finally in water, to remove all excess of silver as the complex thiosulphate. The sensitivity of the test is greatly increased by viewing the dried chromatograms under ultraviolet light (7×10^{-6} g. of thionate over 0·5 cm.^{2} of the developed spot is clearly visible).

Results.—For a photograph of the separation of thiosulphate and thionates by the normal downward technique see Pollard (*loc. cit.*). This shows the double spotting of tetrathionate and the single acid spots obtained for penta- and hexa-thionate. As the amount of double spotting and also R_F values depend on the distance of starting line from the solvent feed, R_F values will not be quoted for this separation.

The solvent finally selected for use with the rear-phase technique was : *tert.*-butanol (15 ml.), acetone (65 ml.), water (20 ml.), potassium acetate (0·5 g., anhydrous) : a higher percentage of potassium acetate led to the decomposition of the higher thionates during the chromatographic run.

With phenol-red in this solvent (0·1%), the R_F value was determined in two different tanks by noting the distances travelled by the phase boundary and the solvent front before the latter reached the clipped pad. After an equilibration period of 10 hr., the values at 17—20° were : tank A, $d = 7$ cm., $R_F = 0{\cdot}39 \pm 0{\cdot}03$; tank B, $d = 5$ cm., $R_F = 0{\cdot}38 \pm 0{\cdot}03$.

The following Table gives distances moved (cm.) by the thio-salts in tanks A and B for : equilibration period = 10 hr., pre-elution period = 24 hr., chromatographing period = 15 hr. The distances of phase boundary from the starting line were : tank A, 26·5 cm., tank B, 31·2 cm.

This distance is measured from the starting line here in order to enable easier comparison of its position with those of other zones.

	Tank A, d = 7 cm.					Tank B, d = 5 cm.				
	S_2O_3''	S_3O_6''	S_4O_6''	S_5O_6''	S_6O_6''	S_2O_3''	S_3O_6''	S_4O_6''	S_5O_6''	S_6O_6''
Tail	2·3	9·5	12·1	14·4	16·9	1·5	8·5	12·0	14·7	18·4
Head	3·8	11·1	14·3	16·0	19·7	2·5	10·1	13·9	16·1	21·4

The separation of the higher thionates may be enhanced either by using a longer run, combined with a long pre-elution period, or by adjusting the length of time of pre-elution and chromatographing so that these species (*i.e.*, penta- and hexa-thionate) move past the phase boundary. For example, in tank B, with 7·5 hours' pre-elution and 15 hours' chromatographing, the phase boundary came between tetra- and penta-thionate, the head and tail movements being :

	S_2O_3''	S_3O_6''	S_4O_6''	S_5O_6''	Phase boundary
Tail	0·5	9·1	16·2	21·5	18·7
Head	2·1	11·5	18·6	24·8	

showing 3 cm. separation between S_4O_6'' and S_5O_6'' instead of 1 cm. as in tank B (first table).

In another run, tetra-, penta-, and hexa-thionate all passed the phase boundary, giving a good separation :

	S_2O_3''	S_3O_6''	S_4O_6''	S_5O_6''	S_6O_6''	Phase boundary
Tail	0·8	11·5	20·5	27·2	33·6	20·5
Head	1·8	13·1	23·5	30·5	36·5	

(Tank B; 7 hours' equilibration, 14 hours' pre-elution, 22·5 hours' chromatographing.)

Thus, by varying the lengths of pre-elution and chromatographing, the separation between any two components may be made greater by the interposition of the phase boundary. Another advantage of the method when applied to these compounds is that no decomposition (as detected by the appearance of zones other than that for the pure thionate spotted) occurs owing to drying the spots on the chromatogram before elution. With the older technique, this occurred except when the chromatogram was placed into the solvent with the spots still wet.

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