The Analysis of Inorganic Compounds by Paper Chromatography. Part VII. A Multiple-spot Phenomenon.*

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Single compounds sometimes give two or more spots on paper chromatograms when mobile phases are used containing an acid different from that corresponding to the anion of the original metal salt. The causes of this phenomenon have been investigated and a detailed account is given of the behaviour of the strontium cation initially present as chloride, nitrate, or acetate with a mobile phase of aqueous butan-1-ol containing acetic acid. Double-spot formation may occur when there is competition for the cation between the original anion and a different anion introduced into the mobile phase, its extent depending upon the concentration and nature of the anion in the mobile phase. Similar double-spot formation can be produced with mobile phases containing a complex-forming agent, *e.g.*, by eluting copper nitrate with a mobile phase containing benzoylacetone.

The production of double zones due to alkaline-earth impurities in the filter paper is also discussed.

It is now well known that single pure substances sometimes give rise to two or more zones on filter paper or cellulose columns. Lester Smith (*Nature*, 1952, **169**, 60), using an aqueous butanol solvent on a kieselguhr column, obtained two solute zones when the butanol was slightly undersaturated with water or contained an excess of water in fine suspension. Consden, Gordon, and Martin (*Biochem. J.*, 1947, **41**, 590) found that metal-salt impurities in paper absorb atmospheric moisture, leading to formation of two zones with amino-acids. Hanes and Isherwood (*Nature*, 1949, **164**, 1107) working with phosphoric esters obtained secondary " ghost " and " shadow " spots which were also attributed to the alkaline-earth and heavy-metal impurities in the paper. These observations led workers in chromatography to pay close attention to (*a*) the purity of the filter paper and (*b*) the water content of partially miscible solvent mixtures.

Many examples are recorded of double-spot formation due to the movement of different species caused by reaction of the original compound with the mobile phase (*e.g.*, Bayley, Bourne, and Stacey, *ibid.*, 1951, **168**, 510; Partridge, *Biochem. J.*, 1948, **42**, 238; Hassall and Martin, *J.*, 1951, 2766). Hahn, Sorkin, and Erlenmeyer (*Experientia*, 1951, **7**, 258) explained the occurrence of three spots when cobalt acetate was run with a solvent containing acetoacetic ester as due to the formation of different " solvated complexes." Erdem and Erlenmeyer (*Helv. Chim. Acta*, 1954, **37**, 2220) similarly recorded three spots for cadmium corresponding to different ammine complexes when the acetate was eluted with a solvent containing propan-1-ol and ammonia. Other examples have been given by Pollard, McOmie, and Elbeih (*J.*, 1951, 466), by Burstall, Davies, Linstead, and Wells (*J.*, 1950, 516), Lederer (*Nature*, 1953, **172**, 727), and Peterson and Reineke (*J. Amer. Chem. Soc.*, 1950, **72**, 3598). Another factor of major importance is the pH of the solvent mixture and of the initial solute solution. Aronoff (*Science*, 1949, **110**, 590) obtained five spots for lysine which were ascribed to different ionic species since the intensity of each spot varied with the pH of the lysine solution; Landua, Fuerst, and Awapara (*Analyt. Chem.*, 1951, **23**, 162) made similar observations for a number of amino-acids. Starting with solutions of potassium or sodium mono- and di-hydrogen phosphates of varying pH values, Curry (*Nature*, 1953, **171**, 1026) obtained separate spots of $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}.

The present paper is mainly concerned with the competition between an added substance and the anion associated initially with the cation.

If, in a chromatographic system, a salt A^+B^- is eluted with a mobile phase containing C^- which can compete with B^- for A^+, then in the process of elution the equilibrium $AB + C^- \rightleftharpoons AC + B^-$ will occur. When the acid H^+C^- is present the

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tendency to form AC will depend on (*a*) the relative affinities of C and B for A, and (*b*) the relative concentrations of C and B, which in turn depend on the p*K* of the acids HC and HB and hence upon the pH of the mobile phase. Although the cation and the anion of a given salt may sometimes move independently, it has also been shown that they may move as ion pairs, depending on the nature of the eluting solvent.

The work described below has shown that two zones are formed when nitrates or chlorides of cations are eluted with aqueous butanol containing acetic acid, one zone being due to the metal nitrate or chloride and the other to the metal acetate. The relative distribution of cation between the two zones depends on (i) the concentration of the acetate ion in the mobile phase and (ii) the concentration of the chloride ion in the original solution of the metal chloride. Only one zone is obtained when the metal acetate is used as starting material.

When copper nitrate is eluted with aqueous butanol containing benzoylacetone, the tendency of the metal to move as its benzoylacetone complex depends on the concentration of complex-forming agent in the solvent. When the solvent contains nitric acid, a lower proportion of the copper moves as the complex. Under certain conditions of pH and concentration of benzoylacetone copper forms two zones, one of the metal nitrate or possibly the monobenzoylacetone complex $[Cu(C_{10}H_9O_2)]^+NO_3^-$ and the other of the bisbenzoylacetone complex $Cu(C_{10}H_9O_2)_2$. Ferric iron can also give double spots in the above type of solvent mixture (see Pollard, McOmie, and Elbeih, *J.*, 1951, 466) and it was thought that the spot of low R_F value was due to a ferric benzoylacetone complex and the spot of high R_F value to a ferrous one. There is now evidence that part of the iron moves as partially covalent complexes such as $[Fe^{III}(C_{10}H_9O_2)]^{2+}$ or $[Fe^{III}(C_{10}H_9O_2)_2]^+$ just behind the acid front and that part moves as $Fe^{III}(C_{10}H_9O_2)_3$ between the acid front and the liquid front.

For quantitative separations these general considerations are important, and we have found them particularly useful in the selection of suitable solvent mixtures in our recent work.

A double-spot phenomenon distinct from that described above is produced by the presence of alkaline-earth impurity, mainly calcium, in the paper itself. Consider a metal chloride MCl, eluted with aqueous butanol–acetic acid. Calcium as impurity at the site of the salt MCl will partially exchange with the metal. The chloride ions will then move on the paper as MCl and as $CaCl_2$, thereby giving two distinct spots. When the impurities are removed by acid-washing of the paper, only the MCl spot is obtained.

EXPERIMENTAL

Competition between Chloride, Nitrate, and Acetate Ions for Strontium.—The general technique of chromatography was that previously described (Elbeih, McOmie, and Pollard, *Discuss. Faraday Soc.*, **1949**, **7**, 183). Whatman No. 1 filter paper was used throughout and strontium was located by the use of sodium rhodizonate.

(a) *Effect of the variation of anion and concentration of cation.* Neutral solutions of varying concentrations of strontium acetate, nitrate, and chloride were eluted with a mobile phase of butan-1-ol (50 ml.), glacial acetic acid (40 ml.), and water (10 ml.). The results are recorded in Fig. 1.

Two zones occurred when two anions were present, and one of these zones corresponded to the position of strontium acetate, while the other zone was due to the nitrate or chloride. The zones due to acetate were the less intense, so that most of the cation had moved under these conditions as the nitrate or chloride. The relative positions and intensities of the zones were unaffected by the initial cation concentration.

(b) *Effect of varying the relative concentrations of chloride and acetate anions in the initial solution.* Mixed solutions of the chloride and acetate of strontium were prepared from the corresponding 0·5N-solutions in the proportions by volume of 10 : 1, 5 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 5, and 1 : 10 severally. Two spots of each solution were chromatographed with the solvent mixture used in (*a*). One of the spots was sprayed with silver nitrate to detect the chloride ion. The position of the chloride spot was the same as that of the strontium spot of low R_F value.

The results recorded in Fig. 2 show that with a relative decrease in chloride-ion concentration the intensity of the upper spot due to strontium chloride decreased, being matched by a corresponding increase in the intensity of the lower zone due to strontium acetate.

(c) *Effect of increasing the chloride-ion concentration in the initial solution of strontium chloride.* Solutions of strontium chloride were prepared by mixing 2·5N-strontium chloride (10 ml.), water (5 ml.), and hydrochloric acid (35 ml.). The concentration of the hydrochloric acid used for the preparation was varied by diluting concentrated acid (*d* 1·16) by factors of 1, 2, 4, 8, 16, and 32. Drops of these solutions were chromatographed as before, and the results are recorded in Fig. 3.

At high chloride-ion concentrations the strontium moved as one spot whose R_F value corresponded to that of strontium chloride, but as the chloride concentration decreased the zone became distended and finally divided into two, the second zone corresponding to strontium acetate. Thus only at lower concentrations of chloride ion did the competing power of the acetate ion in the mobile phase have a significant effect.

Factors affecting the double spotting of strontium.

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

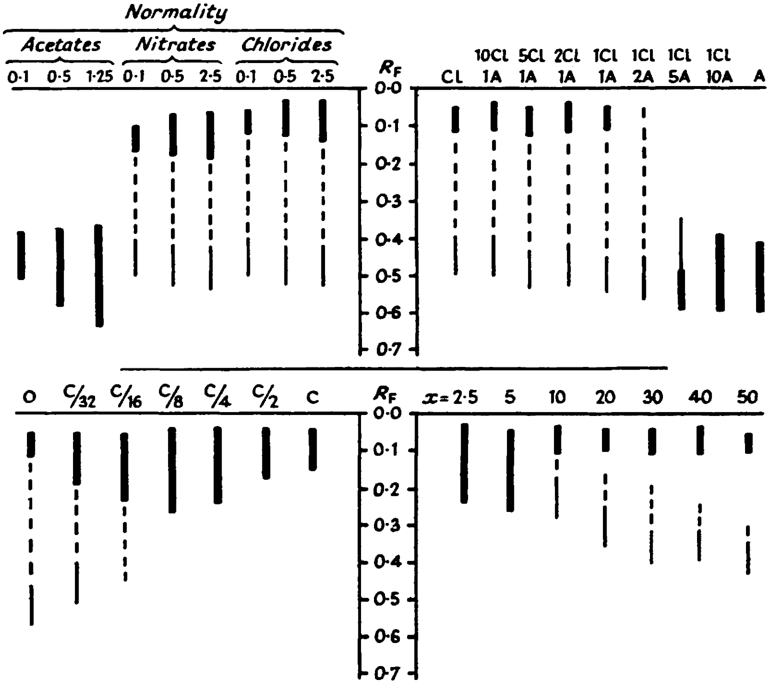

FIG. 1. *The nature of the anion in the salt used.*

FIG. 2. *Relative anion concentrations in mixed acetate–chloride solutions.* Cl *and* A *are the relative concentrations of chloride and acetate in each mixture.*

FIG. 3. *The hydrochloric acid content of the chloride solution. c is the concentration of* (*d* 1·16) *hydrochloric acid used.*

FIG. 4. *The acetic acid content* (*x ml.*) *of the solvent.*

(d) *Effect of increasing the acetate-ion concentration in the mobile phase.* Spots of a 0·5N-strontium chloride solution were eluted with different mobile phases of increasing acetate-ion concentration prepared from butan-1-ol (50 ml.), water (10 ml.), and glacial acetic acid (*x* ml.), where *x* was 2·5, 5, 10, 20, 30, 40, and 50 severally. The results are recorded in Fig. 4.

With low acetate-ion concentration only one zone was formed but this was rather elongated stretching down below the position of strontium chloride. As the acetate-ion concentration increased the zone extended further towards the strontium acetate position until it finally divided into two zones at the higher acetate-ion concentrations. Thus with increase in concentration of acetic acid strontium tends to move more as its acetate and less as its chloride.

(e) *Effect of variation of the water content of the mobile phase.* Spots of 0·5N-strontium chloride were eluted with different mobile phases of increasing water content prepared from butan-1-ol (50 ml.), glacial acetic acid (25 ml.), and water (*x* ml.), where *x* was 0, 1, 2, 5, 10, 20, 30, 40, and 50 severally. Double spots were formed by all the solvent mixtures and hence in this system the phenomenon is not connected with the water content of the mobile phase.

Competition between a Complex-forming Agent (*Benzoylacetone*) *and the Nitrate Ion for* Cu^{2+} *and* Fe^{3+}.—In previous papers (Pollard, McOmie, and Elbeih, *J.*, **1951**, 466; Pollard, McOmie, Stevens, and Maddock, *J.*, **1953**, 1338) the movement of copper in butan-1-ol containing benzoylacetone and nitric acid was shown to depend on the acidity of the solvent. The spot at R_F *ca.* 0·2 was considered to be the benzoylacetone complex but the present work shows that the dibenzoylacetone complex has R_F *ca.* 0·9. Parts (a) and (b) describe the movement of copper on paper chromatograms and part (c) describes further work on the movement of ferric iron. In part (d) liquid–liquid partition experiments with copper and iron in the above solvent system support this work.

(a) *Effect of variation of benzoylacetone concentration on the movement of copper nitrate.* A 0·25N-solution of copper nitrate was used. Copper was detected on the chromatograms with rubeanic acid.

Spots of copper nitrate were eluted with water-saturated butanol containing 10% (w/v) of benzoylacetone and with other solvents obtained by successive dilution of this with equal volumes of water-saturated butanol. In the absence of benzoylacetone, copper moved exclusively at R_F *ca.* 0·1. That this spot was in fact the nitrate was confirmed by the characteristic blue fluorescence observed under ultraviolet light, when the chromatogram was sprayed with "Nitron." When the benzoylacetone concentration was between 0·05 and 0·1%, the copper formed two spots, moving in part to the solvent front as the characteristic green benzoylacetone complex. At higher concentrations of benzoylacetone, the metal moved exclusively in the complexed form.

(b) *Effect of variation of* pH *of solvent on the movement of copper nitrate.* Solvents were prepared by saturating a 0·5% solution of benzoylacetone in butanol with equal volumes of (i) 0·001N-, (ii) 0·01N-, (iii) 0·1N-, and (iv) N-nitric acid. In elutions of copper nitrate with solvents made from (i) and (ii) the metal moved entirely as the benzoylacetone complex. With (iii) and (iv) most of the copper moved as sharply defined spots at R_F 0·1—0·2. With (iv) only a trace of the metal moved as the benzoylacetone complex.

It is most probable that the spot of low R_F is the copper nitrate although the possibility that the $[Cu(C_{10}H_9O_2)]^+$ complex may be present cannot be excluded.

(c) *Movement of ferric iron on paper chromatograms and on cellulose columns.* When ferric salts were eluted on paper chromatograms with butan-1-ol containing 0·5% (w/v) of benzoylacetone and saturated with N-nitric acid (acid front R_F 0·7) two spots were obtained. The spot at R_F 0·9—1·0 was orange-brown and the elongated spot of lower R_F was maroon. The R_F of the lower spot depended on the initial salt, sulphate giving R_F 0·1—0·3, chloride R_F 0·2—0·35, and nitrate R_F 0·3—0·6. This spot is probably due to complexes of the form $[Fe^{III}(C_{10}H_9O_2)]^{2+}2X$, $[Fe^{III}(C_{10}H_9O_2)_2]^+X$, where $X = NO_3^-$, Cl^- or $\frac{1}{2}SO_4^-$. This spot in the acid region immediately gave a blue colour when the dried chromatogram was sprayed with neutral potassium ferrocyanide solution. The spot at R_F 0·9—1·0 in the acid-free region did not give a blue colour with ferrocyanide unless sprayed with dilute mineral acid, and appeared to be due to the fully covalent complex $Fe^{III}(C_{10}H_9O_2)_3$. Ferric salts gave one yellowish spot at low R_F due to the hydrated ion or salt, when eluted with the solvent mixture containing no benzoylacetone.

Further results have been obtained on cellulose columns. When a column was packed with a slurry of cellulose in butan-1-ol and the ferric salt was added to the top and eluted with the butan-1-ol–benzoylacetone (0·5%)–N-nitric acid solvent, zones of different colours were obtained above and below the acid front. The orange-brown zone below the acid front was collected and found to be at pH $\simeq$ 6 while the maroon zone above the acid front was found to be at pH $\simeq$ 2.

A column was packed with the butan-1-ol–benzoylacetone–N-nitric acid solvent mixture and was eluted until the acid front had travelled down the whole column. The ferric salt was then added and eluted with the above solvent mixture and only a maroon zone was obtained, corresponding to the spot of low R_F on paper chromatograms. When a column was packed with butan-1-ol containing only benzoylacetone, and ferric salts were eluted with this solvent, only an orange-brown zone was obtained corresponding to the spot of R_F *ca.* 0·9—1·0 on paper chromatograms.

(d) *Liquid–liquid partition experiments.* Copper and ferric nitrates were separately partitioned in two-phase systems prepared by shaking butan-1-ol containing benzoylacetone with an equal volume of nitric acid of various normalities. The amount of copper or iron in the phases was approximately determined by the colour of the copper or ferric benzoylacetone complex in the upper phase and the copper or ferric nitrate in the lower phase by using a " Spekker " absorptiometer. The approximate amount of copper or iron in the butanol-rich upper phase increased with (i) increase of benzoylacetone concentration and (ii) decrease of normality of acid. Agreement was obtained for the amount of iron found in the upper phase at equilibrium when starting with ferric nitrate or $Fe^{III}(C_{10}H_9O_2)_3$.

The partition coefficients of copper and iron in the same solvent system were sufficiently different to allow complete separation of the benzoylacetone complexes by only 12 transfers in a simplified counter-current distribution train (after Lathe and Ruthven, *Biochem. J.*, 1951, **49**, 540).

Double-zone Formation with Other Cations.—The chlorides of the metal ions, Na, Cu, Ca, Sr, Ba, Mg, Zn, Cd, Hg, Al, Mn, Fe, Co, Ni, were eluted singly with butan-1-ol, acetic acid, and water (5 : 4 : 1). All these cations, except Ca, Fe, and Hg, gave double zones corresponding to the chloride and acetate. The acetate zone had the greater R_F value, but tailed back to the more compact chloride zone. The identity of the metal chloride spots was confirmed as in section (b), on p. 4332. Mercury moved completely as chloride, which dissociated insufficiently to allow of an exchange of anions. Iron was completely converted into complex by the acetic acid, and moved to the solvent front. Failure to detect the less intense calcium acetate spot was obviously due to masking by calcium impurity in the paper. When acid-washed paper was used, all cations except mercuric formed double spots. Therefore, as far as cations were concerned, formation of double-spots appeared to be independent of impurities in the paper. (However, see later reference to iron.)

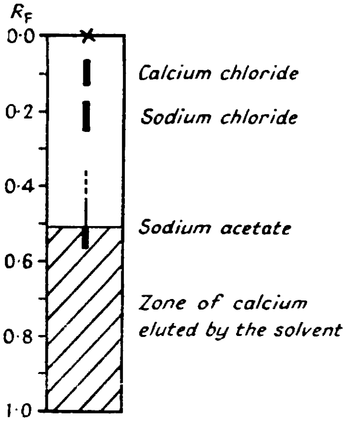

FIG. 5.—*Elution of sodium chloride with butan-1-ol–acetic acid–water* (5 : 4 : 1).

Double-zone Formation with Anions : Alkaline-earth Impurities in the Paper.—In the work described in the preceding section for untreated paper, a well-defined calcium zone, identified with 8-hydroxyquinoline and 4-carboxypyrogallol, was observed in each case (except that of mercury and calcium) at R_F 0·1. The origin of this calcium was obviously the paper itself. The chloride zones of copper, aluminium, sodium, and zinc were well separated from this calcium zone, which was shown on further chromatograms to be calcium chloride. Thus, there were two distinct chloride zones present. Most of the calcium present as impurity in the paper was washed downwards by the solvent, but a certain amount was retained as chloride by anion exchange with the metal chlorides (cf. Fig. 5).

Magnesium is also present in the paper, and moved to the same position as calcium. Mercuric chloride again moved as the undissociated chloride.

When paper was used from which alkaline-earth impurities had been removed by washing with dilute hydrochloric acid, no double chloride zones appeared. With all chromatograms there were faint " secondary " chloride spots which may have been due to traces of heavy metals not removed by acid washing.

Finally, reference must be made to ferric chloride, which gave a single zone of the complex acetate when untreated paper was used, but a chloride and an acetate zone on acid-washed paper. In the former case, chloride ions were removed from the ferric chloride by the calcium in the paper and so all the iron moved as the acetate, thereby appearing at the solvent front.

CONCLUSIONS

From this work it is clear that the R_F value of a metal on a chromatogram depends on the anion of the salt used and on the anion(s) or complex-forming agent in the eluting solvent. With certain combinations of these factors multiple spots can be obtained with the metal moving as two or more species.

For quantitative analysis it is essential that a metal should move as a single species and give a compact spot. In the absence of a complex-forming agent this is most simply achieved by starting with salts in which the anion coresponds to the anion of any acid or salt contained in the eluting solvent. In the presence of a complex-forming agent the acidity should be sufficiently low and the concentration of the complex-forming agent sufficiently high to ensure that the metal finally forms a single spot of the complex.

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