101. *Analysis of Inorganic Compounds by Paper Chromatography. Part I. Movement of Cations with Complex-forming Solvent Mixtures.*

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The behaviour of the 24 cations of the ordinary qualitative tables with a number of different solvent mixtures, alone and with complex-forming reagents has been investigated. Three solvent mixtures, butanol–water containing benzoylacetone, collidine–water, and dioxan containing antipyrine, are suggested for the general scheme of qualitative analysis, details of which are given in Part II (following paper). The existence of new complexes of cations with collidine is reported as a preliminary to further investigation.

Recent developments of paper chromatography have opened up new possibilities for qualitative and quantitative separations of cations and anions. In an earlier communication (*Faraday Soc. Discussions*, No. 7, 1949, p. 183) the possibility was suggested of a new scheme of qualitative analysis using paper chromatography only. For such a purpose, the traditional division of cations into groups has been abandoned, and the analysis depends solely on the different chromatograms obtained with suitable solvent mixtures.

One of the main factors in paper chromatography of inorganic compounds is the distribution between an organic solvent and water. Other workers have shown (cf. Linstead *et al.*, *J.*, 1950, 516 for a complete list of references) that the chlorides, nitrates, and acetates of certain cations are sufficiently soluble in organic solvents to enable movement to take place, but our investigations show that it is often preferable to convert the original salt on the paper into a complex such as that with benzoylacetone.

We have investigated a large number of solvents and organic reagents capable of forming complexes with cations, and have shown that the movement of many cations depends, not only on the complex formed, but also on the solubility of that complex in the organic solvent. The movements of 24 individual cations in the best solvent mixtures are shown in Table I, while details of other eluting mixtures which have special value for certain groups of cations are described in the Experimental section of Part II. Our analyses, described in Part II, are based on three solvent mixtures : butanol–water containing benzoylacetone, collidine–water, and dioxan containing antipyrine.

For the movement of cations there are a number of variable factors, *viz.*, solvent mixture, complexing agent, pH, temperature, nature and state of the paper support; and for the separation of several cations, the possibility of displacement. Until the relative importance of such factors is known, the work must remain empirical and be guided by general principles for the prediction of the movement and separation of cations.

A general spraying reagent for the detection of the majority of cations has already been discussed (see Pollard *et al.*, *loc. cit.*) and consists of a mixture of kojic acid and 8-hydroxyquinoline which reveals the position of cations under ultra-violet light as either fluorescent or dark spots. The sensitivity of detecting reagents is of importance since it partly determines the minimum quantity of any cation which can be chromatographed and detected. The fluorescent reagent is much more sensitive than ammoniacal hydrogen sulphide, which is itself valuable for detecting those cations which form coloured sulphides. Some comparative figures for the sensitivity of these reagents with stationary spots are quoted in Table II, but allowance must be made for diffusion into a larger area when estimating the minimum quantity which can be detected on the chromatogram.

TABLE I.

Movements of cations with different solvent mixtures.

R_F values.

	Butanol containing :							
	dibenzoyl-methane.	benzoyl-acetone.	acetyl-acetone.	acetoacetic ester.	anti-pyrine.	Dioxan containing antipyrine.	Collidine.	Pyr-idine.
Ag	0·18	0·10	0·15	0·18	0·19	0·08	0·78	0·88
Hg^+	0·23	0·24	0·43	0·50	0	0·43	0	0·86
Pb	0·11	0·03	0·09	0·09	0·10	0·15	0	—
Hg^{2+} ...	0·23	0·31	0·43	0·50	0	0·42	0	0·86
Bi	0·15	0·02	0·23	0·20	0·20	0·63	0	0
Cu	0·13	0·22	0·12	0·15	0·17	0·24	0·76	0·85
Cd	0·13	0·05	0·12	0·15	0·13	0·18	0·76	0·87
As	0·42	0·43	0·43	0·45	0·50	0·18	0·65	0·75
Sb	0	0	0·02	0	0	0·65	0·38	0·72
Sn^{2+}	0·73	0·58	0·82	0·70	0·73	0·77	0	0
Sn^{4+}	0·65	0·55	0·81	0·65	0·68	0·58	0	0
Al	0·13	0·03	0·09	0·10	0·09	0·03	0	0
Cr	0·13	0·03	0·09	0·10	0·09	0·01	0	0
Fe^{3+}	0·20	0·95	0·43	0·13	0·14	0·10	0	0
Zn	0·14	0·05	0·10	0·12	0·10	0·08	0·75	0·86
Mn	0·16	0·07	0·11	0·13	0·12	0·09	0·71	0·87
Co	0·13	0·06	0·10	0·12	0·11	0·05	0·74	0·88
Ni	0·13	0·03	0·09	0·12	0·09	0·05	0·76	0·87
Ca	0·11	0·05	0·08	0·12	0·09	0·10	0·52	—
Sr	0·08	0·04	0·07	0·08	0·05	0·04	0·40	—
Ba	0·06	0·02	0·09	0·06	0·04	0·02	0·26	—
Mg	0·11	0·06	0·10	0·13	0·10	0·04	0·65	0·85
K	0·10	0·05	0·10	0·11	0·06	0·03	0·32	—
Na	0·10	0·06	0·10	0·11	0·06	0·04	0·42	—

TABLE II.

Comparison of the sensitivity (in µg.) of hydrogen sulphide and fluorescent spray.

Cation :	Pb.	Cu.	Cd.	Al.	Fe.	Zn.	Co.	Mn.	Ca.	Mg.	K.	Na.
H_2S	0·5	0·5	2·5	—	0·03	—	0·03	—	—	—	—	—
Fluorescent spray ...	0·05	0·03	0·05	0·03	0·03	0·1	0·03	0·03	2·5	0·03	5	5

When the paper is sprayed with our fluorescent mixture and viewed under ultra-violet light, the position of the two liquid fronts (Linstead, *loc. cit.*) is clearly visible. The acid portion is a non-fluorescent region some distance behind the main liquid front, and the space between the acid front and the main liquid front is brightly fluorescent. If the paper is afterwards treated with ammonia, the acid region becomes fluorescent too. At low acidities (*i.e.*, mixtures prepared with 0·1N-acid) the acid front reaches to an $R_F = 0·5$, but at higher acidities (*i.e.*, with 3N-acid) the acid front ($R_F = 0·9$) almost coincides with the main front. It would seem, therefore, that the pH of the solvent mixture will vary as the liquid moves down the paper, and hence the R_F values of cations may vary with the distance moved.

EXPERIMENTAL.

Procedure.—The apparatus, procedure, and preparation of solvent mixtures and cationic solutions have been described in our earlier communication (*loc. cit.*). Tests have been made under the conditions recommended by other workers (Linstead, *loc. cit.*; Longenecker, *Anal. Chem.*, 1949, **21**, 1402), different widths of filter paper (from ½″ upwards) and different types of string being used. We favour, however, a width of paper (about 4 cm. for each cation) which allows the chromatogram to form without interference from the edges of the paper. Provided that adequate precautions are taken to keep the paper strip reasonably taut, the upward movement of the solvent mixture can yield quite as good a chromatogram as the downward movement.

Results.—(I) *The effect of the anion on the movement of the cation.* When the copper salts of the following anions were eluted with a mixture of butanol, water, and acetic acid (5 : 4 : 1), the ions moved in the order indicated : citrate, tartrate and sulphate ($R_F = 0$), chloride ($R_F = 0{\cdot}08$), nitrate ($R_F = 0{\cdot}12$), benzoylacetone complex ($R_F = 0{\cdot}20$), acetylacetone complex ($R_F = 0{\cdot}20$), and acetate ($R_F = 0{\cdot}27$). The quality of the spots was best with benzoylacetone. Addition of 10% of saponin and 5% of glycerol to the solvent mixture improved the definition in the case of the acetate.

It would clearly be inconvenient, even if possible, to begin with complexes such as those with benzoylacetone, and the choice had to be made between the acetate, nitrate, and chloride as the original salt. As is well known, the acetates of many cations show a great tendency to hydrolyse, whilst some chlorides are insoluble. Consequently, we have usually begun with the nitrates and aimed to produce the complexes *in situ* by adding a complex-forming reagent to the solvent mixture.

(II) *The movement of cations in different solvent mixtures.* A very wide range of solvents, with and without the addition of complex-forming reagents, and in the presence of different amounts of acid (or alkali) to produce the right pH, have been tried. Most of the later work has been done with nitrates as the starting material, though initially we used acetates (*Nature*, 1949, **163**, 292). Where it was not convenient to use nitrates, other salts, *e.g.*, the chlorides of Sn^{2+}, Sn^{4+}, and Cr^{3+}, the tartrate of antimony, and an aqueous solution of arsenious oxide, were employed. In all cases the solutions contained sufficient acid to prevent hydrolysis, but it was found inadvisable to have more acid present than was just necessary for this purpose.

Too large amounts of cation tended to overload the paper and to produce chromatographic spots with tails; for example, with copper the best results were obtained by using 0·02 c.c. of 0·1N-copper nitrate, larger amounts of copper producing tailing. In general, the optimum quantity of material to use was 0·02 c.c. of 0·1N-solutions of Ag^+, Hg^+, Hg^{2+}, Pb^{2+}, Bi^{3+}, Cu^{2+}, Cd^{2+}, Al^{3+}, Fe^{3+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, As^{3+}, Sb^{3+}, and 0·02 c.c. of 0·2N-solutions of Ca^{2+}, Sr^{2+}, Ba^{2+}, Mg^{2+}, K^+, Na^+, Sn^{2+}, Sn^{4+}, Cr^{3+}. Much smaller quantities than these are within the limits of detection.

Solvent Mixtures.—(1) *Butanol and water, with different complex-forming reagents.* (a) With nitric acid. The effect of nitric acid has been previously described (Pollard *et al.*, *Faraday Soc. Discussions*, 1949, No. 7, p. 183).

(b) With acetoacetic ester. When using 1 : 1 mixtures prepared with butanol and water or dilute nitric acid (0·1N- to 3N-nitric acid containing 5% of acetoacetic ester), the best separations and most symmetrical spots were obtained with mixtures prepared with N-nitric acid. At lower acidities there was pronounced tailing, which again became the disturbing factor at higher acidities.

(c) With acetylacetone. The best mixture was found to be butanol–2N-nitric acid (1 : 1) and 1% acetylacetone for the general movements, though Cu^{2+} and Cd^{2+} separated when 0·1N-acid was used but did not do so at higher acidities; there was serious forward tailing only with Hg^+, Hg^{2+}, Al^{3+}, and Fe^{3+}. An interesting point is that the tailing produced with iron gradually became more and more pronounced with increasing acidity, and with the mixture using 1N-nitric acid two distinct spots were obtained; the upper one in the acid zone was deep violet (probably ferric complex), the lower one beyond the acid front being orange-yellow (probably ferrous–acetylacetone complex).

(d) With benzoylacetone. The best mixture was found to be butanol–0·1N-nitric acid (1 : 1) and 0·5% benzoylacetone, and since this is the mixture which we use for one of our solvents in our later separations, we have quoted in detail our observations on the effect of changing the acidity of the acid. Table III illustrates the general trend of the effect of varying acidity on the movement when complex-forming reagents are used, which is undoubtedly associated with the stability of the respective complexes at different pH's. The separation of iron into two spots was further investigated by using initial solutions of iron of varying acidity and the mixture quoted above. It was found that with the stronger initial acid solutions the two spots appeared again, and qualitative tests indicated that the one with R_F 0·6 was a ferric, and the other, of R_F 0·95, was a ferrous compound. This illustrates the reducing effect of the cellulose and the change in relative stability of the ferric and ferrous benzoylacetone complexes as the pH changes.

TABLE III.

Solvent mixture.	Observations.
Butanol–water (1 : 1) and 0·5% benzoylacetone	Forward tailing with Hg^+, Hg^{2+}, Sn^{2+}, Sn^{4+}, Al^{3+}, Cr^{3+}, Zn^{2+}, Co^{2+}, Ni^{2+}. One spot for Fe^{3+} (R_F 0·95) with long tailing. Cu^{2+} and Cd^{2+} separate in spite of forward tailing of Cu^{2+}.
As above, but water replaced by 0·05N-aqueous HNO_3	Forward tailing with Cu^{2+} and Al^{3+} only. Tailing of Fe^{3+} decreases. Cu^{2+} and Cd^{2+} still move to different positions.
Ditto, with 0·1N-HNO_3	Best spots and best movements of cations. Fe forms one spot.
Ditto, with 0·2 or 0·3N-HNO_3	Less difference in movements of Cu^{2+} and Cd^{2+}. Long tails with Hg^+ and Hg^{2+}. Fe^{3+} forms two spots, R_F 0·6 and 0·95.
Ditto, with 0·4 or 0·5N-HNO_3	No difference in movement of Cu^{2+} and Cd^{2+}. The lower spot of the Fe^{3+} is smaller. Hg^+ and Hg^{2+} washed down to solvent front with long tails.
Ditto, with 1·0, 1·5, 2, 2·5, or 3N-HNO_3	Cu^{2+} and Cd^{2+} move to the same extent. Tailing forward of Sb^{3+}. Long tails with Hg^+ and Hg^{2+}. One spot for Fe^{3+}, R_F 0·6.

In order to establish that the various complexes were formed during the passage of the solvent, a trial was made on a cellulose column to separate Fe, As, Cu, and Cd, the solvent mixture fractions containing the respective cations being collected and evaporated. The crystals of the iron and the copper compound thus obtained both had m. p.s corresponding to their benzoylacetone complexes namely, 224° and 195°, respectively, while cadmium did not yield crystals of the benzoylacetone complex, and arsenic on evaporation gave a white precipitate which sublimed at 193° and was probably arsenious oxide. Thus, as would be expected, not all the cations move as their benzoylacetone complexes.

(e) With dibenzoylmethane. The best mixture was butanol–2N-nitric acid (1 : 1) and 0·1% dibenzoylmethane, and this gave the best spots obtained with Hg^{+}, Hg^{2+}, Sn^{2+}, and Fe^{3+}; the only other useful feature is that the separation for Cu^{2+} and Cd^{2+} is quite good when *only water* is used to prepare the solvent mixture.

(f) With dimethylglyoxime. This was not found to be so good for the purpose in view. There was hardly any movement of cations unless the acidity was fairly high, and at very high acidity most of the spots had long tails. The best mixture was butanol–N-nitric acid (1 : 1) and 0·1% dimethylglyoxime, which gave reasonable results with Hg^{+}, Hg^{2+}, and Sb^{3+}.

(g) With antipyrine. A mixture of butanol and N-nitric acid (50 : 50) containing 1% of antipyrine produced movements of some cations, *e.g.*, Ag^{+}, Pb^{2+}, Bi^{3+}, Cu^{2+}, Cd^{2+}, As^{3+}, and Sn, without tailing, but on the whole the movements and separations were not as good as those for antipyrine in dioxan.

(h) With other complexing reagents. Catechol, protocatechuic acid, 8-hydroxyquinoline, and ammonium thiocyanate could not be used with nitric acid, since they readily oxidised and were generally not satisfactory.

The addition of pyridine or collidine as complexing reagents produced no valuable results, except that butanol, 0·1N-nitric acid, and collidine (5 : 4 : 1) produced the only separation of Cd (R_F 0·24) and Zn (R_F 0·06). Usually there was movement accompanied by pronounced tailing.

(2) *Collidine mixtures.* The collidine–water mixture is heterogeneous at room temperature. Mixtures with 50% of 0·1, 0·2, 0·3, and 0·4N-nitric acid cause a rise in temperature, but those with 0·6N-nitric acid raise the temperature and also lead to the formation of a homogeneous mixture which does not separate into two layers on cooling, as the others do. With these mixtures very good separations of many cations were obtained and are recorded for the best mixture (collidine and 0·4N-nitric acid, 1 : 1) in Table I. The observations on the effect of acidity are quoted in Table IV.

TABLE IV.

Solvent mixture.	Observations.
Collidine with water or 0·1N-HNO_3 (1 : 1)	Tailing in Cu^{2+}, Cd^{2+}, Sb^{3+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mg^{2+}, K^{+}, Na^{+}.
Ditto, with 0·2 or 0·3N-HNO_3	Tailing is less for Cu^{2+}, Sb^{3+}, Zn^{2+}, Mn^{2+}, and Co^{2+}. Gradual concentration of spots for most cations.
Ditto, with 0·4N-HNO_3	Best spots with almost no tailing at all.
Ditto, with 0·5N-HNO_3	Tailing forwards with Hg^{+}, Hg^{2+}, Cu^{2+} and Co^{2+}.
Ditto, with 0·6N-HNO_3 (see above)	Washing down of most of the cations to the solvent front or excessive tailing.

A series of qualitative test-tube experiments with the 24 cations and collidine shows that only the cations which move in the collidine mixture form complexes with collidine that are soluble in that solvent. Only one collidine complex, the azide $(C_7H_9N)_2,Cu(N_3)_2$, has been recorded (Cirulis and Straumanis, *J. pr. Chem.*, 1943, **162**, 307). The formation and properties of these complexes are being further investigated.

(3) *Pyridine mixtures.* Pyridine, like dioxan, is completely miscible with water, so only one layer was formed when it was mixed with different quantities of water or acid. The best mixture consisted of 60% of pyridine and 40% of water, and this gave movement with all cations except Pb, Ca, Sr, Ba, K, and Na where, if there was movement, it was accompanied by considerable tailing.

Pyridine, alone or with from 1 to 6% of concentrated nitric acid, either gave considerable forward tailing or washed the cations to the solvent front. Somewhat similar results were obtained with mixtures of 90% and 10% of water or different concentrations of dilute nitric acid (from 0·5 to 2N.), but in this case two spots appeared for Hg^{+}, Hg^{2+}, and Cd^{2+}. Pyridine with 2% or 4% of ammonium thiocyanate added gave movement with Ca, Sr, Ba, K, and Na, accompanied by much tailing, whereas other spots were either washed down or did not move at all.

The R_F values for Pb, Ca, Sr, Ba, K, and Na were unsatisfactory owing to tailing. The quality of the spots with pyridine was not as good as those with collidine.

(4) *Dioxan mixtures.* Dioxan, which is miscible with water, was not satisfactory when used alone, but a mixture of 100 c.c. of *pure* dioxan, 1 g. of antipyrine, 1 c.c. of concentrated nitric acid, and 2·5 c.c. of water was found to move a number of cations with good separation and concentrated spots. The R_F values are recorded in Table I, and this solvent mixture is the third one used in our scheme. For some cations, *e.g.*, Al and Sb, the R_F value varies with the initial concentration of the cation.

(III) *The effect of the filter-paper on the movement of the cations.* (a) Different types of filter-paper. Other workers, particularly Hanes and Isherwood (*Nature*, 1949, **164**, 1107), when separating phosphoric esters, have stated that the type and purity of the paper will determine the concentration of the spots and the movement of the compounds. Experiments with Whatman No. 1, No. 54, No. 541 for chromatography, and purified No. 1 (*i.e.*, treatment with 8-hydroxyquinoline in aqueous alcohol, followed by thorough washing in aqueous alcohol and drying), using the usual cation solutions and the butanol–

water–benzoylacetone mixture showed that: (i) There was no general improvement in the concentration of spots and the movement was the same for all filter-papers. (ii) The purified No. 1 had no " ghost " spots remaining on the starting line, although they are present in very faint shadows with No. 54 and No. 541—slightly less than with No. 1. (iii) The only marked improvement we were able to observe was that the solvent mixture ran more quickly over No. 54, and there was slightly less tendency for tailing of the spots.

(b) Treatment of the filter-paper. Attempts were made to change the condition of the paper by: (i) Oxidising it with sodium periodate and bromine water to convert some of the $CH_2{\cdot}OH$ groups of the cellulose into CO_2H groups; papers so treated exhibited increasing tendency to stop movement of the cations and produced excessive tailing where movement did occur. (ii) Treatment with nitric acid: (*a*) Papers soaked in different concentrations of dilute nitric acid (5—20%) for ½ hour, washed, and dried, gave slightly better movements, but tailing was not completely prevented. (*b*) Papers soaked in mixtures of nitric and sulphuric acids (5% H_2SO_4 and 10% HNO_3; or 7% H_2SO_4 and 14% HNO_3) for 10 hours, then washed with distilled water and dried, led to the stopping of the movement of most of the spots, though there was a slight tailing forward. (iii) Treatment with succinic anhydride to increase the number of CO_2H groups present on the paper also led to an increasing tendency for the cations not to move, or to a slight tailing forward.

Our conclusion from these experiments was that for ordinary analytical work there were no great advantages to be gained in any treatment of the paper which we have tried.

In some early experiments we soaked Whatman No. 1 paper in methyl alcohol for several hours to lessen the water content of the paper, and then tried to chromatograph the cations with a butanol–glacial acetic acid (9 : 1) mixture. In this case there was hardly any movement of the cations, but with untreated Whatman No. 1 and water only as the solvent, all the cations were washed down to the solvent front with long tailings. When a mixture of butanol, water, and acetic acid (5 : 5 : 1) was employed as the solvent mixture, the cations moved. This indicates that water has an important function in this type of separation.

(IV) *Effect of temperature on the separations, spots, and solvent mixtures.* The overall effect of temperature was investigated by using the three general solvent mixtures based on butanol, collidine, and dioxan mentioned earlier, and placing the whole tank in a temperature-controlled air cupboard at 16°, 25°, or 38°. In general, the positions to which the cations move do not seem to change with temperature, but the increased rate of flow of the solvent mixtures enables quicker separations to be achieved especially at 25°. At too high a temperature (*i.e.*, 38°) there is considerable transverse diffusion of the spots and excessive tailing. With a fairly volatile solvent like dioxan, the increased evaporation of the solvent from the paper, if the tank is not completely saturated with vapour, has the effect of slowing the movement. It is therefore not easy to determine the real effect for dioxan.

Further investigation of the temperature effects will be carried out later.

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