163. *Chromatographic " Spot " Tests.*

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A piece of filter-paper, when treated with an adsorbent, preferably peptised alumina, and incorporating a developer, will act like a chromatographic column towards a solution containing constituents suitable to the developer, and exhibit coloured zones, specific to the constituent, solvent, developer, and pH only. These zones are suitable for comparative analysis. The order of such zones is not the chromatographic series, for the mobility of the constituent in question may be that of an ion, molecule, or newly formed complex.

Flood's formula (*Z. anal. Chem.*, 1940, **120**, 327) has been found to hold in most cases.

It has been shown by Flood (*loc. cit.*) that filter-paper, when treated with sodium aluminate in such a manner that alumina is precipitated on the fibre, exhibits selective adsorption and behaves with inorganic solutions in a manner similar to a chromatographic column as shown by Schwab (*Angew. Chem.*, 1940, **53**, 39). Papers may, however, be treated with other adsorbents to acquire these properties. For instance, blotting-paper, *i.e.*, paper containing fuller's earth, has been found satisfactory, provided it be smooth, for loose fibres, by creating capillary forces in different directions, cause considerable variation in the behaviour of the paper towards ions or constituents normally travelling in a direction away from the point of feeding. Starch paper was also found suitable in several instances, but papers containing silica gel have not been found effective. The adsorptive properties of the paper are not impaired by dyeing the whole sheet by immersion in an organic dye. It was, therefore, considered possible to include in the adsorbent paper a reagent showing coloured complexes with the constituents of a solution to be analysed. Here several distinct types of visible adsorption have to be considered, *i.e.*, those involving (*a*) coloured insoluble complexes, (*b*) coloured soluble complexes, (*c*) selective adsorption of a dye by a constituent, (*d*) constituents assuming distinctive colours of their own on separation.

Into categories (*a*) and (*b*) would fall a solution of ferromolybdenum in dilute acid. When such a solution is treated with 8-hydroxyquinoline in acetic acid solution, only the molybdenum complex is precipitated, the iron complex being soluble in acid solution. A paper treated with alumina and oxine solution and dried, when spotted with the ferromolybdenum solution will develop a yellow (Mo) central zone and a black (Fe) outer zone, the area of each being indicative of the relative amounts present. A different ferromolybdenum solution at the same pH will give an absolutely comparable result. The molybdenum here is precipitated instantly and is adsorbed as a complex, displacing the iron, which travels in ionic form and only forms the visible complex on drying.

As an example of category (*c*), a paper treated with alumina on which a mixture of amyl acetate and ethyl alcohol containing a trace of fluorescein has been spotted will show a coloured central zone and a colourless outer ring. The alcohol and the acetate travel freely, and adsorption would have taken place at the same levels without the presence of the dye; the latter, being insoluble in amyl acetate, remained with the alcohol. Again, good and comparable results were obtained on repetition.

The most suitable paper for constant results was obtained by using a colloidal solution of alumina prepared by peptisation of a solution of sodium aluminate with acetic acid (Weiser, *J. Physical Chem.*, 1920, **24**, 525), and air-drying the paper after immersion. In some cases, however, another adsorbent has to be used. For example, starch papers have to be used in tests specific to alkali metals; such papers, soaked in violuric acid solution and dried, will give distinctive and comparable results for mixtures of sodium, potassium, and ammonium. Here it was noted that, although the presence of any of the above did not influence the chromatographic picture of the others, which were separated by clear blank zones, yet the pH has to be very strictly controlled, as this reagent forms different complexes in neutral and in acid solution, the results from neutral solutions being very difficult to observe, owing to less intensive colours.

For simple investigations, Flood's formula zone radius $= f(\log c + A)$, where $c =$ concentration and A is a constant for the paper, is valid. It will, however, be found that, as the pH influences the size of all zones, a specific constant for each constituent at a definite pH and for a specific paper has to be found empirically. When only comparative results are required, *i.e.*, when the course of a reaction is to be continuously observed, the knowledge of this constant is unnecessary. It was found that the slightest variation in the preparation of the papers influenced the constant considerably. The dependence on the pH varied with the sensitivity of the reagent or developer incorporated.

If a solution contains constituents A and B, three drops (say) of the solution are placed on a suitable paper at regular intervals during the reaction, giving two ring-like zones, the first one (for A) being bounded by radii r_1 and r_2, and the second for B by radii r_3 and r_4 (if B $=$ Mo on an oxine paper, $r_4 = 0$). If the two rings are not separated by a blank zone, $r_2 = r_3$. If the initial solution is of known concentrations a and b, respectively, then

$$a = (r_1{}^2 - r_2{}^2)K_a\pi \text{ and } b = (r_3{}^2 - r_4{}^2)K_b\pi$$

and the values of K_a and K_b may be calculated. If, however, the first test is denoted by suffix α, and the next one by β, the variation may be obtained by direct measurement :

$$a_\alpha/a_\beta = (r_1{}^2{}_\alpha - r_2{}^2{}_\alpha)/(r_1{}^2{}_\beta - r_2{}^2{}_\beta)$$

Further, from the above, we have

$$a/b = (r_1{}^2 - r_2{}^2)K_{a/b}/(r_3{}^2 - r_4{}^2), \text{ where } K_{a/b} = K_a/K_b.$$

For illustration, the following table shows some separations which are easily achieved on such papers. The adsorbent was peptised alumina in all cases except that of Na/K/NH$_4$, for which it was starch.

Constituents.	Developer solution.	Remarks.
Fe/Mn	Formaldoxime hydrochloride	3% Of ferromanganese, just acid. Expose to NH$_3$ vapour after drying
Fe/Mo/V/Ti	5% Oxine in 10% acetic acid	1% Metals, just acid
Ni/Co	Nitrosonaphthol and dimethylglyoxime, aqueous	Any concentration, strongly acid
Na/K/NH$_4$	Violuric acid, aqueous	Buffered acetic acid solution
Ketones, aldehydes, and cyclic carboxyl compounds	2 : 4-Dinitrophenylhydrazine, 2% in acetic acid	Alcoholic solution or solvent-free mixtures
Cu/Ni/Fe/Cr (VI)	α-Benzoinoxime, 2% in alcohol	Nearly neutral. Expose to NH$_3$ vapour when dry

EXPERIMENTAL.

Selection of Paper.—The paper was tested by dissolving 5 g. in 5 c.c. of sulphuric acid and heating the solution to fuming (hot plate) with sufficient nitric acid to destroy organic matter. The mixture was diluted to 100 c.c. and filtered. After neutralisation with ammonia the solution should give no colour change or precipitate with oxine solution. A paper having passed this test is dipped into water in such a way that one inch is submerged and 3 inches remain clear : if the paper is suitable, the water should be drawn up to 2 inches above the surface by capillary forces within one minute.

Preparation of Paper.—The paper is dipped in a peptized alumina solution (as above) or into fairly concentrated hot starch solution. Alternatively, it is dipped in sodium aluminate solution, dried, and dipped in sodium carbonate solution. In each case it is washed for 5 hours at 20—25° with stirring, soaked in the chosen developer, washed by immersion in water, and air-dried at room temperature.

Analysis of Ferromanganese.—A paper prepared with peptised alumina and formaldoxime hydrochloride solution is used. The test solution is made to contain about 3% of metal and is neutralised with ammonia to incipient precipitation. A standard stain with a metal of Fe/Mn = 0·01081 gives r_1 = 39·17 mm., r_2 = 34·00 mm., r_3 = 28·8 mm., r_4 = 0. This stain is obtained by using two drops of the solution, allowing it to dry on a flat surface, and exposing it to ammonia vapour. From the above, $(r_1{}^2 - r_2{}^2)/(r_3{}^2)$ = 0·456, *i.e.*, $K_{Fe/Mn}$ = 0·0237. A stain of metal of unknown composition gave the radial ratio as 0·363, *i.e.*, Fe/Mn = 0·363 × 0·0237 = 0·0086. The analytical result showed the sample to have a ratio Fe/Mn = 1 : 112·5 = 0·00889.

2 : 4-Dinitrophenylhydrazones.—A paper impregnated as above and dipped in a 1% solution of the reagent in alcohol to which 5% acetic acid had been added was dried. A solution containing 10% each of methyl hexyl ketone, benzaldehyde, and acetaldehyde in ether was spotted on. Three distinct zones were obtained with benzaldehyde in the centre and acetaldehyde in the outer zone. One after the other of the constituents was reduced in area till it eventually disappeared. The zones not affected stayed constant, while those affected were reduced in area in proportion to the composition of the solution.

Alkali Metals.—A paper is treated with starch and then with a solution of violuric acid according to Erlenmeyer (*Helv. Chim. Acta*, 1941, **24**, 878). An aqueous solution containing 5% of sodium acetate, 5% of ammonium chloride, and 5% of potassium chloride, to which 2% of acetic acid has been added, is spotted on. A spot with an inner blank zone was followed by a red sodium zone (r_4 = 0·95, r_5 = 1·50 cm.), a pink potassium zone ($r_4 = r_5$, r_3 = 1·90 cm.), a blank zone, and a blue ammonia zone (r_2 = 2·45, r_1 = 2·475 cm.). From these the following areas are calculated : Na = 4·24, K = 4·27, NH$_4$ = 4·71 cm.². By exactly halving the concentrations of ammonium chloride and potassium chloride in the same solution, the radii measured became r_6 = 1·25, r_5 = 1·70, $r_4 = r_5$, r_3 = 1·890, r_2 = 2·14, r_1 = 2·315 cm. These give the following areas : Na = 4·24, K = 2·15, NH$_4$ = 2·40 cm.².

Ferromolybdenum.—A paper impregnated with peptised alumina and 8-hydroxyquinoline was used. 2 G. of the metal were powdered, and warmed with nitric acid till no more brown fumes appeared. 10 C.c. of concentrated hydrochloric acid were added and the mixture was evaporated to a small bulk. 15 C.c. of 18N-sulphuric acid were added, and the mixture was evaporated to fuming. After being cooled and boiled with 50 c.c. of water, the mixture was filtered, and to this solution ammonia was added till a turbidity appeared which was cleared again with sulphuric acid. On spotting, this solution gave an outer zone of black (Fe), separated from an inner yellow spot (Mo) by a blank zone. For this solution and paper, the factor $K_{Fe/Mo}$ had been found to be 0·81. A sample known to contain 73·2% of Mo, gave the factor Fe/Mo = 0·346 (actual : 0·35).

Molybdenum and Vanadium.—On a paper prepared with alumina and 8-hydroxyquinoline, comparable results have been obtained at very definite pH limits. At pH values 8—9 the vanadium is in the centre, being insoluble and instantly precipitated. At pH values 8—4 the results for the same solution stayed constant only at exactly the same pH. At stronger acidities the pH seemed no longer to exert such a strong influence, as is expected from the solubility of the two complexes.