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Quantitative Cusp Paper Chromatography

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A relative assessment of the main methods of quantitative chromatography is given. The theory and practice of cusp chromatography (a new variant of precipitation chromatography, developed by the writers) are discussed, and examples are given of the determination of cations and anions, singly and in mixtures, and over a very wide range of concentrations. 38 references.

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I. INTRODUCTION

A short review of the development of precipitation chromatography, beginning with the work of its originators (Gapona and Gapon[1,2]), and also a discussion of the theory and many interesting practical applications of precipitation chromatographic analysis, can be found in the monograph by Obshanova and coworkers[3], and more recent developments in a review article by Lur'e[4]. The potential uses of partition chromatography are discussed in a recently published symposium[5].

The bands of precipitation chromatograms are formed by chemical reactions under well defined and reproducible conditions. The direct proportionality between the size of the bands in precipitation chromatograms and the amount of material being analysed has been stressed by many workers[7-14]. This feature of precipitation chromatography distinguishes it from partition and from sorption chromatography, and offers (in principle) a simple and reliable method of quantitative determination by measuring the size of the bands in precipitation chromatography. The most convenient form of the method from this point of view is precipitation chromatography on paper, and in particular one of its variants which allows the bands to be obtained in the shape of cusps[15]. However, much of the earlier work was aimed at the study of chromatograms of a different type[7,16-18]. No attempt was made to determine the optimum conditions for the formation of cusp chromatograms, and even the reason for the formation of bands in the shape of cusps was not discussed. In general, cusp chromatography was ignored. A recent symposium[5] deals with quantitative chromatography by physicochemical methods: in the present review we shall discuss the direct determination of the amount of material in the band by measurements of the size of the bands (and, in particular, the height of the cusps[6]).

II. QUANTITATIVE CHROMATOGRAPHY BY MEASUREMENT OF THE SIZE OF BANDS

There is general agreement[4] that sorption and partition chromatography on columns do not lend themselves to quantitative determinations from measurements of the size of the bands, mainly because these bands have indeterminate stoichiometries and geometries. The concentration of the substance being analysed in the bands, and the size and shape of the bands themselves, show a complicated dependence on the composition and concentration of the solution under study, on the composition and specific surface area of the sorbent or of the carrier—stationary phase mixture, and also on the dynamics of the process (which can be further complicated by diffusion in the bulk of the sorbent or through the porous carrier). On the other hand, paper chromatography and thin-layer chromatography offer the possibility of quantitative analysis directly from the chromatogram (e.g. by scanning the bands with a densitometer or with a radioactivity detector if suitable tracers are used). However, we cannot yet "rely on the reproducibility and precision of the results"[5]. This is not surprising, since the composition of the stoichiometrically indeterminate bands depends on the chromatographic conditions and on the relative order and duration of the various steps. These uncertainties disappear completely when we turn to precipitation chromatography on paper: bands consisting of precipitates have a definite stoichiometry and (in the case of paper chromatography) a very small and constant thickness. Simple calibration (by determining the width of a standard band) is sufficient for precise measurement of the amount of material present in the precipitate: this presupposes that the sample for chromatography is deposited on the paper as a band of constant width, or (preferably) that the band is produced in the form of a cusp with a constant, narrow base[15].

Kokk and Aleskovskii[19,20] were led by similar considerations to use thin threads as columns of the wick type, and were able to claim enhanced sensitivity for quantitative column chromatography.

The paper specially produced for chromatography has a constant density. If a uniform, stationary layer of precipitant is deposited on such a paper, quantitative chromatography of substances which form sparingly soluble compounds with the precipitant becomes possible.

An immobile layer of precipitant—one of the main requirements of quantitative chromatography—is obtained by impregnating the paper with a relatively insoluble precipitant. Soluble precipitants should be avoided, because they are partially eluted during the chromatographic separation and the reproducibility of the results is thereby impaired. For this reason, the more recent work[22-24] on cusp paper chromatography was done with paper impregnated with sparingly soluble precipitants, in contrast to the earlier work[15,20,21], in which the cusps had been obtained on paper impregnated with soluble reagents. This small improvement has resulted in satisfactory

reproducibility of the cusps and has established cusp chromatography as a strictly quantitative method.

A uniform distribution of precipitant in the bulk of the paper is obtained using appropriate impregnation conditions, established experimentally. The uniformity of the precipitate in a column of inert carrier has been demonstrated[25,26] by scanning the column (anhydrous alumina) with a counter after precipitation of silver iodide containing radioactive iodine. The uniformity and the attachment of the precipitate on paper are, obviously, no worse than in a column, as shown by the excellent reproducibility of quantitative measurements on paper impregnated with sparingly soluble precipitants. The layer of deposit is believed[25] to consist of charged colloidal particles which are held on the surface of the carrier by electrostatic attraction. Recrystallisation, leading to growth of the primary crystals, is also thought of occur.

The exact size of the band is ultimately determined by the particle size of the precipitate. Hence it is important to adopt chromatographic conditions favouring the formation of crystallites of a reproducible size.

In order to ensure that the whole of the sample deposited on the chromatographic paper comes into contact with the impregnant (precipitant), the primary chromatogram is eluted in various ways[3] with a solution free from the ions being analysed. For example, the paper containing the sample can be steeped in water or in an aqueous solution of glycerol to elute the primary chromatogram.

Another important condition for good results in quantitative paper chromatography is a large difference in solubility between the impregnant and the precipitates of the elements being analysed. Evidently, the impregnant should be more soluble than the precipitates. Under these conditions, Lur'e concludes[4] from a consideration of the dynamics of sorption that "we can expect a discrete distribution of the precipitates in eluted chromatograms in the form of individual bands, practically free from impurities of other components", as other workers had previously reported from experimental work.

The solubility of the precipitates in the various bands can be increased by varying the acidity of the chromatographic solution or by increasing the concentration of complexing agents in solution. It follows that the pH must be optimised and controlled, and in general the concentration of all the constituents of the solution which influence the solubility of the precipitates in the chromatographic bands must also be controlled.

Yet another important condition to be satisfied in quantitative chromatography is very rapid formation of the precipitates: this must be faster than the rate of penetration of the solution through the paper during elution[27].

The difference in the degree of utilisation of the precipitant observed during chromatographic separation of different ions, e.g. zinc sulphide during the separation of silver and copper ions, is ascribed to the different rates of the corresponding replacement reactions: "this difference is greatest when the difference in solubility product between the reactants and the final products is greatest[28]".

Kulaev points out[16,29] that the size of the bands of various precipitates varies in direct proportion with the following factors: 1) the solubility product of the sparingly soluble precipitate; 2) the concentration of the original solution; 3) the drop volume of the original solution. It is also "determined by the conditions under which the precipitate is formed, particularly since the last stages of formation of the precipitate take place in the narrower regions". Kulaev also stresses that "other conditions being the same, the width of the band ... depends only on the solubility product of the sparingly soluble compound", a fact which he ascribes to the dependence of the rate of migration of the precipitation front upon the solubility product. Lur'e[4] does not accept this argument, and suggests that the width of the band is determined by the difference in reaction rates of the components of the solution with the precipitants, which is directly related to the difference between the solubility products of the impregnant and of the precipitate in the final chromatographic band.

Obviously, this controversy can be resolved by a study in depth of the mechanism of precipitation chromatography.

True precipitation chromatograms on paper are often obtained in simple spot tests[30,31]. For example, 0.05—0.25 μg of copper can be detected on paper impregnated with rubeanic acid in the presence of a large excess of cobalt and nickel. These elements produce separate, differently coloured annular bands on the paper. According to Feigl[31], unsized paper is simply an assembly of compressed fibres. The mixture to be analysed is separated by diffusion and adsorption in the capillaries of the paper. The precipitate formed in the interstices between the paper fibres has a fine-grained structure: it can migrate with the solution, cover the surface of the fibres, and eventually plug up the capillaries. In this way a small amount of precipitate can cover a relatively large area of paper, thus making the spot test on paper more sensitive than on a smooth tile or in a test-tube. Because of their small particle size and large surface area, the precipitates formed in the capillaries of the paper are more soluble than the same precipitates formed in test-tubes.

The sensitivity of spot tests can be increased by treating the paper with gelatine. The main difference between paper chromatography and spot-test analysis resides in the method of applying the sample and in the subsequent treatment of the chromatogram. Kulaev[16-18,29] suggests the following procedure[16].

Filter-paper is impregnated with 1—4% solutions of sodium silicate, hydrogen phosphate, or arsenate, or with solutions of potassium ferrocyanide or chromate. The excess of solution is allowed to drain off, and the paper is air-dried whilst still hanging in a vertical position. Samples of the solutions to be analysed are deposited from a capillary dropping pipette or a microburette (drop volume 0.025 ml) on a 65 mm square piece of the paper. The drop should be allowed to fall on the paper from a height "no more than 5 mm from the surface". A circular spot is formed on the paper. This primary chromatogram is eluted by adding 5—6 drops of water, each drop being allowed to spread fully before the next drop is added. The colourless bands are developed by spraying a suitable developer, in repeated small doses, onto the dried chromatogram.

When a mixture of two or more elements is chromatographed, the band corresponding to the least soluble of the elements remains in the centre as a circular spot; the more soluble precipitates surround the central spot in concentric rings disposed in order of increasing solubility. In Kulaev's experiments the impregnated paper contained a small excess (a factor of 2—3) of precipitant over the amount required to react with the ions under study (mercury, iron, bismuth, chromium, aluminium, cadmium, lead, silver, copper, zinc, manganese, cobalt, nickel, barium, strontium, and several others). The width of the band was measured to the nearest 0.1 mm. The procedure used in cusp chromatography, though outwardly similar, is fundamentally different.

This method of quantitative chromatography was first proposed[15] for the determination of copper and nickel on paper impregnated with a 0.1% aqueous solution of sodium diethyldithiocarbamate or a 0.1% alcoholic solution of rubeanic acid (for copper) or with a 1% alcoholic solution of dimethylglyoxime or rubeanic acid (for nickel). A drop of a solution of copper or nickel salt of known concentration is deposited from a calibrated capillary pipette (1.5 μl volume) on a piece of paper impregnated with the appropriate reagent. The edge of the paper below the drop is immersed in a 14% aqueous solution of glycerol contained in a beaker covered with a glass plate. The solvent is drawn into the paper: it entrains any ions which have not yet reacted, and transports them upwards, forming a cusp-shaped band.

The height of the cusp was found to be linearly related to the concentration of the ion being determined and to the amount of precipitant on the paper. By analogy with the spot-test technique, an attempt was made to increase the sensitivity of the determination by treating the paper with a 0.013—0.05% solution of gelatin after the deposition of the precipitant. This amount of gelatin has a negligible effect on the speed of travel of the solvent, but it gives a sharper and more deeply coloured spot.

Kokk and Aleskovskii[21] subsequently developed a method of determining as little as 5×10^{-2}% of copper in micro-quantities of cadmium selenide (0.50—1.00 mg) on grade B chromatographic paper (density 85 g m^{-2}) impregnated with sodium diethyldithiocarbamate. The relative error of the determination was not greater than 17%; cadmium ions interfere, and they had to be removed from the cadmium selenide.

The same authors[19] determined submicrogram amounts of copper in micro-quantities of cadmium selenide (0.5—1.0 mg) on cotton, artificial silk, and natural silk fibres.

Analysis on fibres is probably the most attractive variant of the quantitative chromatography of elements. Whereas in paper chromatography only the thickness of the bands is constant, it is possible to standardise both the thickness and the width of the bands by using fibres of constant diameter, and furthermore the sensitivity of the method can be increased dramatically by choosing very narrow fibres and by measuring the length of the bands under a microscope. Thus, amounts of copper as small as 5×10^{-4} μg were determined in the above experiments[19]. However, paper chromatography is simpler and more convenient for visual estimation, and therefore cusp chromatography is more likely to find general application.

III. THE CUSP PAPER CHROMATOGRAPHY METHOD

The potential advantages of cusp chromatography as a method of chemical analysis and physicochemical research have been demonstrated in the writers' laboratory[22-24,27,32-34].

1. Chromatographic Procedure

In the preferred method[23,27,32], sheets of chromatographic paper (density 90 g m^{-2}) are cut into 6×15 cm strips. Each strip is drawn through a solution of impregnant of the required concentration. In order to produce a precipitate on the fibres of the paper, the sheet is also drawn through a solution containing a 1.5—2-fold excess of precipitant (as compared to the concentration of the ion to be analysed in the test solution).

The impregnated paper is dried; for work at maximum sensitivity the paper is impregnated with a solution of gelatin and dried again.

Paper containing a stoichiometric amount of precipitant ion is prepared by introducing the precipitant ion first, followed by the exchangeable ion (grade "O" paper). The paper can also be impregnated in the reverse order: the exchangeable ion first, followed by the precipitant ion, i.e. the ion of opposite sign to the ion under test. Due to sorption effects, this procedure gives a paper containing an excess of the precipitant ion (grade "R" paper).

Two pencil lines are drawn on the impregnated paper: an immersion line (0.5 cm from the edge of the paper) and a start line (2—2.5 cm from the edge of the paper). Four or five pencil marks are made on the start line 1.0—1.5 cm apart. Solutions of the test ions are deposited on these marks by touching the paper with calibrated glass pipettes of 0.001—0.002 ml capacity, drawn out to a conical point and ground to a smooth tip.

The paper strips with their spots of test solution are dried in the air: the resulting primary chromatograms are eluted in a 500 ml beaker containing 50 ml of a 12% aqueous solution of glycerol, or water, into which the paper is dipped up to the immersion line. The chromatogram is held in the beaker by means of a glass plate and Petri dish of special design, which ensures that the paper cannot touch the bottom or the walls of the beaker. As the solvent seeps into the paper, it entrains the ions under test which have not yet reacted and displaces them upwards. At the end of the experiment, the precipitate (which does not move with the solvent) forms a trace in the shape of a regular cusp. The elution time is 30—40 min: when elution is complete the chromatogram is air-dried for 10—15 min and the height of the cusp is measured with a pair of dividers and a ruler. The reproducibility of the measurements is good, and in practice only 1 or 2 measurements are needed once the procedure has been established.

In some chromatograms the band fails to develop fully, and only the initial part of the cusp is visible. The apex of the cusp must then be found by extrapolation, by producing the sides of the cusp until they intersect.

2. Conditions Under Which Cusp Chromatograms Are Formed

The chromatographic conditions have been fully discussed by one of us[27].

a. The primary chromatogram

We assume that the paper is uniformly impregnated with a sparingly soluble, microcrystalline precipitant for the ion under test. The volume of the sample of solution, and the thickness and density of the paper, are constant.

As soon as the solution from the capillary pipette begins to wet the paper, precipitate begins to form in a cylindrical region of increasing diameter d (see Fig. 1), and solution denuded of the ions being chromatographed (say, A_a^-) begins to filter outwards through the cylindrical surface of this region.

Some of these ions are transferred to the precipitate by an exchange reaction with the impregnant, and some are adsorbed onto the precipitate. Thus, the ions being chromatographed are held within a cylinder of diameter d, as though it were a chromatographic column, consisting of a very thin layer of freshly deposited $M_u A_a$ spread over

the surface of the paper fibres and therefore in a highly reactive state. As the concentration of the ion under test in the original solution increases, the diameter of this column also increases until it becomes comparable with the diameter of the water spot d_w. Initially, as long as the ions in excess of the stoichiometric requirements of the impregnant are held only by sorption forces, the quantity of both adsorbed and precipitated ions is proportional to the volume of the column, and therefore the total amount a of ions under test is a function of the volume of the column and hence of the square of its diameter. We can write

$$d = D\sqrt{a} \qquad (1)$$

where D is a constant coefficient. However, as d approaches the value d_w, the processes by which the test ion is accommodated in the spot become more complicated.

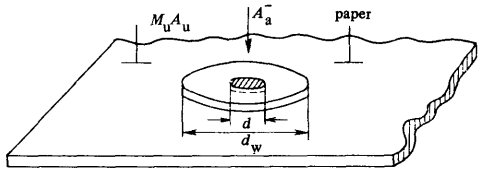


Figure 1. Spot formation in the primary chromatogram.

When the whole of the impregnant within the spot has reacted to give a precipitate (e.g. $M_uA_a.nM_aA_a$, where nM_aA_a denotes the amount of adsorbed ions in excess of stoichiometry), increasing amounts of the free compound (M_aA_a) of the test ion begin to accumulate within the volume of the spot. This effect complicates the relationship between the diameter of the spot and the amount of ions under test. Finally, in the limit the spot size becomes constant, for obvious reasons:

$$d = d_w = \text{const.} \qquad (2)$$

Evidently, within the range of a values to which Eqn. (1) can be applied we can use the spot diameter to characterise the sorption of ions by their precipitates, and thus investigate precipitation reactions quantitatively.

The quantitative study of the sorption of ions by their precipitates in the form of primary bands in paper precipitation chromatograms has been discussed[27] by reference to the following observations. With very small samples of ions, just above the limit of detection, the spot diameter of the primary chromatogram is always smaller than the diameter of a water spot deposited on the paper with the same capillary pipette.

Thus, in experiments with a 2 μl pipette the diameter of the water spot was 0.70 cm. The diameter of a spot of silver iodide (containing 0.5 μg of iodine) was only 0.50 cm, and copper diethyldithiocarbamate gave spot diameters of 0.55 and 0.10 cm for copper contents of 0.5 μg and 0.01 μg respectively. As the content of the chromatographic ion in the sample is gradually increased, the spot diameter in the primary chromatogram increases almost exactly as expected on the assumption that the ion is adsorbed on its precipitate. The departure from a straight-line relationship at small values of a can be ascribed to sorption of the test ion on the precipitate formed by a double decomposition reaction between the test solution and the impregnant, and not to sorption of the test ion on the paper[27].

b. The secondary chromatogram, and cusp formation

As stated above, the secondary chromatogram is obtained by eluting the primary chromatogram with a solvent (e.g. aqueous glycerol, or pure water). Elution is carried out simply by allowing the solvent to seep into the paper. The spot containing a precipitate, formed in the primary chromatogram, may or may not retain an excess of the precipitant. Hence we can define two special cases[32]:

$$\text{I. } a \leqslant uS; \quad \text{II. } a > uS$$

where u is the titre of the paper (mg-equiv cm^{-2}), and S is the area of the spot of precipitate (cm^2).

In the first case the whole of the sample of ions within the spot is bound in the precipitate as M_uA_a or M_aA_u, but in the second case only part of the test ions is bound (by precipitation or sorption on the precipitate), and the remainder is in its original form of the soluble compound M_aA_a and its ions. In this latter case the stoichiometric excess of ions will be washed out of the circular spot by the eluting solvent and carried along the interstices of the paper, where it will meet a fresh supply of the impregnant M_uA_u. Under these conditions, the precipitation of the ion under test will extend beyond the area defined by the primary spot.

The inequality $a \leqslant uS$ defines the system studied by Kulaev[16-18,29] and others, whereas the inequality $a > uS$ defines the conditions for cusp chromatography. In the first case the size of the bands on the chromatograms is determined mainly by the solubility of the precipitates and by diffusion of ions, and is related to the concentration of the ions only through the diffusion effect. In the second case ionic diffusion plays only a secondary role: the main contribution comes from convection, i.e. the transport of ions by the flow of solution.

Depending on the magnitude of the difference

$$a - uS = \Delta \text{ mg-equiv.}, \qquad (3)$$

a more or less extensive trace of the ion under test is formed on the paper in the direction of motion of the solvent, and under favourable conditions this trace has a regular cusp shape.

A simple geometrical construction is sufficient to show that cusp formation must follow from the circular shape of the original spot and from the uniform transport of ions under test along the plane of the paper by the flow of eluent (Fig. 2). The quantity of ions transported by the solvent from different section of the circular spot decreases from the centre towards the periphery, in inverse proportion to the length of the chord parallel to the direction of flow. It has been shown experimentally[27] that regular cusps can be obtained by allowing solvent to seep into the primary chromatogram only if the original spot is truly circular, and then only if the moving solvent front remains strictly parallel to the edge of the paper immersed in the liquid.

3. Fundamental Aspects of Cusp Chromatography

As stated above, the height of the cusp increases with the difference $\Delta = a - uS$. Obviously, it must decrease as the titre of the paper (u) increases. This relationship can be expressed by the formula

$$h = \frac{k_1\Delta}{u} = \frac{k_1(a - uS)}{u} \quad (4)$$

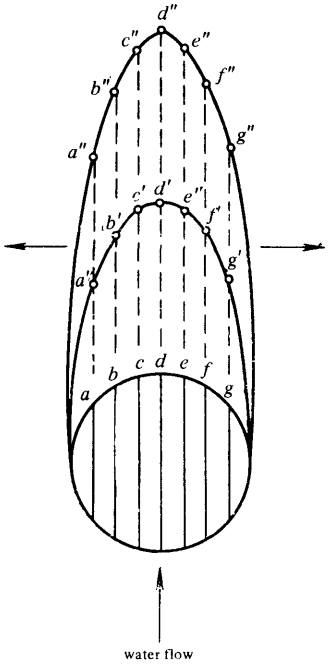


Figure 2. Cusp formation. The points a, b, c, d, e, f, g correspond to the condition $a \leqslant uS$; the points marked with the single prime and the double prime correspond to a small excess and a large excess, respectively, of the test ion in comparison with uS.

This equation was deduced[27] by assuming that the cusp can be approximated by a triangle of height h connected by its base to a semicircle of diameter a (equal to the diameter of the circular spot in the primary chromatogram).

The titre of the paper is approximately equal to the ratio of a to the height of this triangle:

$$u \simeq \frac{2a}{dh} \text{ mg-equiv. cm}^{-2} \quad (5)$$

If the sample volume and the titre of the paper remain constant, the product uS is constant to a first approximation, and the height of the cusp is linearly related to the quantity of ions in the sample:

$$h = \frac{k_1 a}{u} - k_1 S = k_2 a - B \quad (6)$$

Hence

$$a = \frac{h + B}{k_2} \quad (7)$$

As an example of the application of this linear relation, we may quote the results obtained for the chromatographic analysis of iodide ions on paper impregnated with silver chloride[22-24], and also of silver ions on "O" grade paper impregnated with lead diethyldithiocarbamate[27,32]. From the same data, plotted as $h = f(u)$ for constant a, we obtain a family of hyperbolae.

It is possible to calculate[27] the concentration P of the precipitant in solution which should be used to impregnate the paper for the chromatographic analysis of a mg-equiv of an ion. The calculation assumes that the strip of paper retains V ml of the solution of impregnant, and that the whole of the precipitant (G grams) contained in this volume remains in the paper after impregnation. For dilute solutions we can write

$$\frac{G \cdot 100}{V} = P, \%,$$

whence

$$G = \frac{P \cdot V}{100}$$

and the titre of the paper is $u = \dfrac{G.10^3}{\text{equiv wt.}S_p}$ mg-equiv cm^{-2}, where S_p is the area of the strip of paper in cm^2. Inserting the value of G into this formula we obtain

$$u = \frac{PV \cdot 10^3}{\text{equiv. wt.} \cdot S_p \cdot 100} = \frac{10PV}{\text{equiv. wt.} \cdot S_p} \quad (8)$$

Then, using formula (5), we obtain an expression relating the percentage concentration of the solution of impregnant to the amount of ions under test and to the cusp height:

$$u \approx \frac{2a}{dh} \simeq \frac{10PV}{\text{equiv. wt.} \cdot S_p}, \text{ whence}$$

$$P = \frac{2a \text{ equiv. wt.} \cdot S_p}{10 \cdot Vdh} = \frac{0.2 \text{ equiv. wt.} \cdot S_p}{Vdh} \quad (9)$$

All the quantities in formula (9) are either known or easily determined experimentally.

Formulae (5) and (8) have been tested[27,35] by direct analytical determinations of the titre of the paper.

Having established the titre of the paper, known amounts of iodide, bromide, and thiocyanate were chromatographed. The results were used to calculate the titre of the paper by formula (5), and an independent estimate of the titre was obtained by inserting the volume V of impregnant solution and the area S_p of the paper into formula (8).

The titre of the paper calculated by formula (8) agrees with the results of chemical analysis within experimental error, and the values obtained by the approximate formula (5) are also in fairly good agreement.

For thiocyanate these values are closer to the titres obtained by direct chemical analysis than for iodide and bromide. This is because, as shown by chemical analysis of the precipitates in the halide cusps, the atomic ratio of iodide and of bromide to silver increases and tends to 1.5 as the titre of the paper and the amount of halide are increased, suggesting the formation of a complex of the type $K_2Ag_2I_3$. For thiocyanate, on the contrary, the ratio decreases from 1.0 to 0.7. Thus, the effective equivalent of the halide was not the same as the assumed value. Hence we conclude that formula (5) should include a correction equal to the atomic ratio of the amount of precipitant ion (for example, M_u^+) to the amount of the chromatographic ion:

$$u \approx \frac{2a}{dh}\left(\frac{M_u^+}{A_a^-}\right) \approx \frac{2a}{dh} \cdot n \quad (5a)$$

The values given by formula (5a) for the titre of the paper agree somewhat better with the results of chemical analysis.

It is worth noting that, if the titre of the paper is known, the composition of the precipitate in the cusp can be determined from chromatographic data only (d, h, and a): this is a very rapid method of determining the composition of complexes in the solid state.

4. Sensitivity Considerations

The smallest amount of an element which can be estimated by chromatography is that which corresponds to the smallest reproducible cusp height h_0: we find[27] that $h_0 = d$, where d is the diameter of the primary circular spot. Hence, the sensitivity of determinations by the cusp method is approximately the same as that of the corresponding spot tests. In general, the reproducibility of analytical procedures becomes acceptable when the sample size is at least one order of magnitude greater than the limit of detection: we therefore recommend[27] a choice of conditions leading to a minimum cusp height of 10 spot diameters.

The sensitivity of the cusp method is influenced by a number of factors, the most important being those which determine the solubility of the precipitate in the cusp. They include: the pH of the solution, the concentration of complexing agents in solution, the ionic strength and temperature of the solution, the volume of the eluting solution held by the paper, and some others.

Obviously, we should consider first of all the volume of solution taken up by the paper, which directly determines the amount of impregnant which dissolves and hence the amount of precipitate formed by the ion under test.

When the solution used to elute a primary precipitation chromatogram rises to a height H in a strip of paper of width L, the compound of the test ion M_uA_a (e.g. AgI) can form a cusp only if it is present in sufficient amount to saturate a volume $V = H.L.\delta$ cm^3 (where δ is the thickness of the paper) without going completely into solution.

If 1 ml of a saturated solution of M_uA_a contains a_p mg-equiv of the test ion, the paper strip will contain $V.a_p$ mg-equiv of the ion in solution. Hence, if the sample contains a mg-equiv of the test ion, the amount which goes into the precipitate is

$$a - V \cdot a_p = a^* \text{ mg-equiv.} \qquad (10)$$

The height of the cusp is correspondingly decreased, i.e. the dissolution of the precipitate lowers the sensitivity of the determination. Let a^* equal u_0S (the smallest quantity of precipitate of the test ion which can be detected on paper). Inserting this quantity into Eqn. (10) we obtain $a - V.a_p = u_0S$. Hence we arrive at an expression for a_0, the sensitivity of the chromatographic analysis:

$$a_0 = Va_p + u_0S \qquad (11)$$

In the simplest cases, for example in the determination of halide and thiocyanate ions on paper impregnated with silver chloride, we can assume

$$a_p = [A_a^-] \text{ g-ion litre}^{-1}$$

From the equation for the reaction $M_uA_a \rightleftharpoons M_u^+ + A_a^-$ we can write

$$[A_a^-] = \frac{SP_{an}}{[M_u^+]},$$

but $[M_u^+]$ is the solubility of the impregnant, which in the present case can be written $[M_u^+] \simeq \sqrt{SP_{imp}}$. Thus, $[A_a^-] = \dfrac{SP_{an}}{\sqrt{SP_{imp}}}$. Inserting the resulting value of $a_p = [A_a^-]$ into Eqn. (11) gives $a_0 = \dfrac{V.SP_{an}}{\sqrt{SP_{imp}}} + u_0S$. Turning to Eqn. (10), we note that for $a = V.a_p$ the quantity a^* equals 0, i.e. analysis becomes impossible when

$$a = \frac{V \cdot SP_{an}}{\sqrt{SP_{imp}}}$$

or, in general,

$$a_{lim} = \frac{V \cdot SP_{an}}{S_{imp}} \qquad (12)$$

where a_{lim} is the smallest detectable amount of the test ion in the sample, which is a constant for one particular ion and impregnant under a given set of conditions, and S_{imp} is the solubility of the impregnant.

Thus, the sensitivity of the method is higher, other conditions being equal, the smaller the solubility product of the analytical precipitate and the higher the solubility of the impregnant.

One of us[27] has tabulated the values of a_{lim} calculated by Eqn. (12) for thiocyanate, bromide, and iodide on paper impregnated with silver chloride or silver oxide.

It is evident from Eqn. (11) that the sensitivity of detection of ions is determined by their limiting amount a_{lim}, with an additive correction given by the product u_0S. (The latter is greater than a_{lim} by many orders of magnitude for very insoluble compounds of the ion being analysed, but for relatively soluble compounds it does not differ from a_{lim} very significantly.) Hence it is clear that the sensitivity of detection of thiocyanate, bromide, and iodide must increase in the order of decreasing a_{lim}, with very similar values for thiocyanate and bromide. According to our results[27], the sensitivity should be higher on paper impregnated with Ag_2O than on paper impregnated with AgCl. Since the solubility of Ag_2O is greater than that of AgCl†, this conclusion is consistent with the theoretical prediction that the sensitivity must increase with the solubility of the impregnant if other conditions remain constant. Experimental confirmation has been obtained[22-24] that the sensitivity of detection of thiocyanate and halide ions is higher on paper impregnated with Ag_2O than on paper containing silver chloride.

5. Range of Detection of Ions

It can be seen from Eqn. (4) that the height of the cusp can be controlled within any prescribed limits by varying the titre u of the paper. Hence, we can assign to every range of a values a value of u such that the cusp height h will fall within the prescribed limits, e.g. such that the largest cusp shall fill a strip of chromatographic paper 15 cm long. It may be convenient to divide[27] the very wide range of analytical levels of detection found in practice into a number of more limited ranges, each corresponding to a factor-of-ten variation in the size of the sample (e.g. from 10^{-6}—10^{-5} mg-equiv for range 1 to 10^{-3}—10^{-2} mg-equiv for range 4). This allows us to estimate

† The solubilities of AgCl and Ag_2O are 1.2×10^{-5} and 1.9×10^{-4} mg-equiv ml^{-1} respectively.

the amount of ions in each sample under the most favourable conditions, ensuring that the paper is always impregnated with the optimum amount of precipitant.

Clearly, by increasing the titre of the paper we can lower the cusp height by the amount needed to ensure that the cusps for high as well as for low concentrations of the test ion are contained within a chromatogram of a given size. As regards analytical range and sensitivity, the best results are obtained at some particular values of the titre (e.g. 2.0×10^{-5} and 3.3×10^{-5} mg-equiv cm^{-2} for silver and copper respectively).

These general rules for micro-concentrations of test ions can easily be extended to the determination of high concentrations of ions by the cusp method. We have described[27] the chromatographic analysis of concentrated solutions of potassium iodide and bromide up to saturation conditions, with test samples containing up to 500—800 μg of halide.

6. Chromatographic Analysis of Mixtures of Ions

It has been shown experimentally[27] that when the test solution contains a mixture of ions capable of reacting with the impregnant to form a precipitate, a cusp consisting of the least soluble precipitate is formed first of all, followed by the other precipitates in order of increasing solubility.

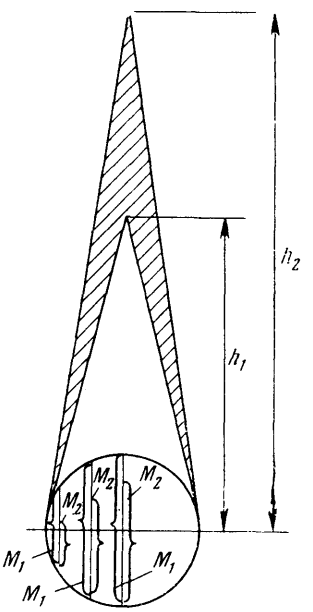


Figure 3. Double cusp formed by two different ions M_1 and M_2 with the same impregnant. (The precipitate formed by M_1 is less soluble than the precipitate formed by M_2.)

Hence, it was suggested that the equality $a_1 + a_2 = uS_1 + uS_2$ (or in general $\Sigma a = u\Sigma S$) can be applied to precipitates of a given type, where a_1 and a_2 are the amounts of two different ions in the sample, u is the titre of the paper, and S_1 and S_2 are the areas of the corresponding bands.

It is evident from Fig. 3 that

$$S_2 \simeq \frac{1}{2} h_2 \cdot d - \frac{1}{2} h_1 \cdot d = \frac{1}{2} d (h_2 - h_1),$$

whence

$$a_2 \simeq \frac{ud}{2} (h_2 - h_1),$$

and putting $\frac{ud}{2} = m$ we obtain

$$a_2 = m(h_2 - h_1) \qquad (13)$$

i.e. the amount of precipitate for the more soluble ion is proportional to the difference in height between the cusps for the more soluble and the less soluble ion.

By a similar argument it can be shown that the amount $a_{(n+1)}$ of an ion mixed with n other ions which form more insoluble precipitates with the same impregnant is proportional to the difference in cusp height

$$a_{n+1} = t \cdot \Delta h \qquad (14)$$

Experiment shows[27] that this relationship applies only when the titre of the paper has been optimised with respect to the ion which forms the most soluble precipitate.

Various mixtures of anions and cations have been analysed by applying these considerations. Thus, mixtures of traces and of relatively large amounts of halides have been chromatographically separated and estimated on paper impregnated with silver oxide.

Precipitation chromatography can be applied in qualitative as well as quantitative analysis. For example, chloride and bromide impurities in potassium iodide can be separated and determined by cutting out the different constituents of the cusp. The effectiveness of this new method of analysis has been confirmed by separating a complex mixture of cations (silver, copper, lead, and cadmium).

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Recent studies[22-24,27,32] of the conditions under which cusp-shaped bands are formed in precipitation paper chromatography have led to the development of a new quantitative method for the determination of ions: cusp paper chromatography. This method differs from existing chromatographic procedures in that it relies on accurate measurements of length, and furthermore it depends on stoichiometric relationships like the classical methods of quantitative analysis. It differs from the latter, however, in being a true chromatographic method, and therefore it offers all the advantages of speed and simplicity which one associates with chromatographic analysis.

Cusp chromatography is a particularly fast method, and is suitable for use in works laboratories and under field conditions, for example in the wet assay of mineral deposits. It can be used to determine anions and cations, in solutions varying in concentration from very dilute to almost saturated. Mixtures of ions as well as single ions can be detected by cusp chromatography, and the method can tolerate the presence of many extraneous substances. 40—50 tests can be done in one hour, and since each test requires only 1—2 μl of solution, a complete analysis can be done on a drop of solution (0.01 ml). The sensitivity of the method is comparable to that of conventional spot tests: for example, 0.2 μg or 1.6×10^{-6} mg-equiv for iodide and 0.02 μg or 6.3×10^{-7} mg-equiv for copper. The relative error of determinations within the same concentration range is 2—10% (for example, 2—7% for iodide ions).

It is important to stress the fact that this is a genuine micro-method of analysis, since only 1—2 μl of solution are needed for each determination. The precision (usually 1.5—11%) can be improved by calibrating the capillary used to deliver the sample (or by using a micro-syringe or micro-pipette), and also by measuring the cusp height to 0.1 mm or better. Evidently, this method of determining micro-quantities of ions can be combined with some method of concentrating trace elements (for example, the "sinking particle" method[36,37]); in this way, the contents of a 1-litre sample of solution can be concentrated into a volume of 0.1 ml, and the range of detection can be extended by 3—4 orders of magnitude.

Another advantage of the method is the possibility of obtaining quantitative information from imperfectly formed cusps, by graphically extending the edges of the cusp and using their intersection to locate the missing apex.

Paper chromatography by the cusp method offers an almost instantaneous method of qualitative and quantitative analysis of large groups of ions (and their mixtures) having closely similar properties: for example, chloride, bromide, and iodide. The elements can be separated by cutting the complex cusp into bands, corresponding to the individual elements.

The theoretical relationships between the height of the cusp and the amount of test ion, and between the height of the cusp and the titre of the paper, and also the formulae for calculating the titre of the paper, have all been confirmed experimentally for both anions and cations. The deviation from linearity of the initial portion of the function $h = f(a)$ has been shown to be due to sorption of the ion by its precipitate (formed by a double decomposition reaction with the impregnant), which is more significant at low concentrations of the test ion. The simple relationship between the diameter of the circular spot formed in the primary chromatogram and the amount of test ion in the sample applied to the paper can be used to study the sorption of various ions by the precipitate formed by their reaction with the impregnant. The observation that a small spot formed within the thickness of the paper acts as a miniature chromatographic column will be applied in future studies of the sorption properties of many precipitates. In the analysis of thiocyanate and halide ions on paper impregnated with silver oxide or silver chloride the cusp height was found to be unmistakably related to the concentration of the silver nitrate solution used to impregnate the paper, and therefore also to the titre of the paper.

However, in the analysis of silver, copper, and some other cations on paper impregnated with metal diethyldithiocarbamates a simple relationship was found only with "O" grade paper, prepared as described above. This observation led to the following empirical rule for the preparation of paper for selective cusp chromatography: the paper should be impregnated first with the precipitant ion, followed by the ion of the same sign as the test ion.

The rule is confirmed by experiments on cadmium and lead, chromatographed singly or in admixture with silver and copper on "R" grade paper, i.e. paper impregnated with lead diethyldithiocarbamate "not according to the rule". Although neither lead nor cadmium should develop a stain on paper impregnated with lead diethyldithiocarbamate, cusps were obtained on this paper, but not on "O" paper (impregnated with lead diethyldithiocarbamate in stoichiometric proportions). This anomaly can be explained only by sorption and coprecipitation phenomena on the "R" grade paper. Evidently, quantitative observations of coprecipitation phenomena can be made on chromatographic paper of this type.

Starting from simple considerations, we have established a relationship between the sensitivity of the determination and the solubility of the precipitates in the simplest case of an exchange reaction between the singly charged test ion and the (more soluble) compound of the singly charged precipitant ion. The resulting formula states that the greater the solubility of the impregnant, and the smaller the solubility of the precipitate formed on the paper by the test ion, the lower is the loss of test ions by dissolution in the eluent and therefore the more quantitative is the formation of the cusp. This explains why the sensitivity increases in the order of decreasing solubility product when a series of ions is analysed on paper of a given titre containing a given impregnant.

Thus, previous assumptions that the size of the bands in precipitation chromatograms is determined by the relationship between the velocity of the precipitate front[16,38] or the rate of precipitation[14,28] on the one hand, and the solubility product on the other hand, cannot be upheld. The same conclusion can be reached, even more simply, by noting that the size of the bands cannot be determined by the rate of any particular process since equilibrium is established when the bands have grown to their full size.

REFERENCES

1. E. N. Gapona and T. B. Gapon, Dokl. Akad. Nauk SSSR, 60, 41 (1948).
2. E. N. Gapona and I. M. Belen'kaya, Kolloid. Zhur., 14, 323 (1952).
3. K. M. Ol'shanova, V. D. Kopylova, and N. M. Morozova, "Osadochnaya Khromatografiya" (Precipitation Chromatography), Izd. Akad. Nauk SSSR, Moscow, 1963.
4. A. A. Lur'e, Uspekhi Khim., 37, 104 (1968) [Russ. Chem. Rev., No. 1 (1968)].
5. E. Shellard, "Paper and Thin-layer Quantitative Chromatography" (Translated into Russian), Izd. Mir, Moscow, 1971.
6. V. B. Aleskovskii and V. N. Aleskovskaya, Nauch. Trudy Leningrad. Tekhnol. Inst. imeni Lensoveta, No. 15, pp. 3—70, Leningrad, 1972.
7. H. Flood, Discuss. Faraday Soc., 7, 190 (1949).
8. M. Milone, G. Cetine, and F. Ricca, Ann. Chim. (Italy), 43, 652 (1953); Chem. Abs., 5720 (1954).
9. M. Muto, Nippon Kagaku Zasshi, 85, A11, 147 (1964); Chem. Abs., 61, 15326a (1964).
10. W. Kielczewski and M. Schneider, Chem. Analit., 11, 111 (1966).
11. N. Ganchev and K. Koev, Trudy Vys. Inst. Narodnostopanstvo, Varna, No. 37, N4, pp. 25—30, 1965.
12. N. Ganchev and Ya. Todorova, Trudy Vys. Inst. Narodnostopanstvo, Varna, No. 37, N4, pp. 35—38, 1965.
13. Ts. Todorova and T. Zhecheva, Zavod. Lab., 32, 417 (1966).
14. K. Koev, Zhur. Anal. Khim., 9, 1053 (1964).
15. E. S. Boichinova and V. B. Aleskovskii, Trudy Leningrad. Tekhnol. Inst. imeni Lensoveta, 48, 94 (1958).
16. F. N. Kulaev, Zhur. Anal. Khim., 14, 278 (1959).
17. D. A. Vyakhirev and F. N. Kulaev, Trudy Kom. Anal. Khim. Akad. Nauk SSSR, 6, No. 9, 527 (1955).
18. F. N. Kulaev, Izv. Vys. Ucheb. Zaved., Khim. i Khim. Tekhnol., 7, 904 (1964).

19. Kh. Yu. Kokk and V. B. Aleskovskii, Izv. Vys. Ucheb. Zaved., Khim. i Khim. Tekhnol., 7, 19 (1964).
20. Kh. Yu. Kokk, Thesis, Leningrad, 1968.
21. Kh. Yu. Kokk and V. B. Aleskovskii, Izv. Vys. Ucheb. Zaved., Khim. i Khim. Teckhn., 7, 564 (1964).
22. V. N. Aleskovskaya and V. B. Aleskovskii, Kratkie Soobshch. Nauch.-Tekhnol. Konferentsii Leningrad. Tekhnol. Inst. imeni Lensoveta, p. 45, 1968.
23. V. N. Aleskovskaya and V. B. Aleskovskii, Zhur. Anal. Khim., 24, 1216 (1969).
24. V. N. Aleskovskaya, Sbornik Trudov Leningrad. Inst. Tekst. i Legkoi Prom. imeni Kirova, 10, 84 (1969).
25. A. A. Lur'e, Thesis, Moscow, 1963.
26. A. A. Lur'e, Zhur. Anal. Khim., 21, 929 (1966).
27. V. N. Aleskovskaya, Thesis, Leningrad, 1969.
28. A. A. Lur'e, Zhur. Fiz. Khim., 41, 25 (1967) [Russ. J. Phys. Chem., No. 1 (1967)].
29. F. N. Kulaev, Zhur. Anal. Khim., 13, 172 (1958).
30. N. A. Tananaev, "Kapel'nyi Metod" (The Spot-test Method), Goskhimizdat, Moscow, 1954.
31. F. Feigl, "Spot Tests", (Translated into Russian), Inostr. Lit., Moscow, 1962.
32. V. N. Aleskovskaya and V. B. Aleskovskii, Zhur. Anal. Khim., 25, 243 (1970).
33. V. N. Aleskovskaya and V. B. Aleskovskii, Kratkie Soobshch. Nauch.-Tekhnol. Konferentsii Leningrad. Tekhnol. Inst. imeni Lensoveta, p. 58, 1969.
34. V. N. Aleskovskaya, Kratkie Soobshch. Nauch.-Tekhnol. Konferentsii Leningrad. Tekhnol. Inst. imeni Lensoveta, p. 59, 1969.
35. I. P. Kalinkin and V. B. Aleskovskii, Izv. Vys. Ucheb. Zaved., Khim. i Khim. Tekhnol., 6, 553 (1963).
36. V. B. Aleskovskii et al., "Kontsentrirovanie i Opredelenie Mikroelementov" (Concentration and Determination of Trace Elements), Izd. Leningrad. Tekhnol. Inst. imeni Lensoveta, Leningrad, 1957.
37. V. B. Aleskovskii et al., "Kontsentrirovanie i Opredelenie Mikroelementov" (Concentration and Determination of Trace Elements), Goskhimizdat, Leningrad, 1958.
38. F. N. Kulaev, "Khromatografiya, Ee Teoriya i Primenenie" (Principles and Applications of Chromatography), Izd. Akad. Nauk SSSR, Moscow, 1960, p. 450.

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