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Note

A simple apparatus for simultaneous chromatography and electrophoresis on thin layers

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Two-dimensional combinations of chromatography and electrophoresis on paper or thin layers have been applied to a variety of mixtures. Because of the different mechanisms of the two separations, the two-dimensional run sometimes leads to a particular distribution of the spots on the chromatogram. Therefore this technique not only results in improved separations, but could also in addition give important information for the identification of unknown components of the sample. This aspect is especially important for the analysis of complicated mixtures of trace amounts of radioactive compounds, such as those formed by nuclear recoil processes^{1,2}.

The application of the two separations consecutively is usually a lengthy procedure, particularly as intermediate drying of the plate is necessary in order to change the liquid phase. Consequently, this procedure is of limited value for the analysis of mixtures of relatively unstable compounds. In these instances, however, the simultaneous application of the two techniques could be considered*. Such a method was reported as early as 1948 by Haugaard and Kroner³ for the separation of amino acids on paper sheets. Separations of some inorganic ions on paper were reported by Strain and Sullivan⁴ in a continuous or discontinuous procedure using a cell of simple construction. Aqueous solutions were used as solvents, resulting in poor chromatographic separations, which were based mainly on differences in adsorbability. The first thin-layer application was described by Behrens⁵, who separated radioactive iodine compounds, which were too volatile to be analyzed by the consecutive method. However, the simple experimental arrangement was found to have severe disadvantages when applied to our systems. Applying a voltage across a thin-layer plate in a solvent trough causes the current to flow through the liquid in the trough instead of through the thin-layer material. The construction of the electrical contact between the voltage supply and the layer presents additional problems, such as charring of the cellulose, local evaporation of the solvent and distortion of solvent flow and front.

* Such a technique can be regarded as electrochromatography in the true sense of the word. To distinguish between this method and the established consecutive technique, we propose the term "chromatophoresis".

EXPERIMENTAL

The above problems were surmounted by using the apparatus shown in Fig. 1, which is constructed of Perspex of 2 mm thickness and consists of a 20×20 cm plate on which eight electrolyte reservoirs and two graphite electrode blocks are mounted. The chamber can be used in conjunction with any type of thin-layer material coated on a glass or plastic support. The thin-layer plate and the chamber are placed horizontally on a cooled surface; the cooled metal block of a Desaga thin-layer electrophoresis chamber is suitable. The solvent penetrates the thin-layer material from the eight separate reservoirs, which are provided with an 11 mm diameter sintered glass disk (G4) of 3 mm thickness, 2 mm of which protrude from the bottom of the reservoir. The eight circular fronts develop from the disks as soon as the chamber is placed on the layer merge and form a regular front within a few minutes. The flow-rate of the solvent, which depends not only on the properties of the solvent but also on the porosity of the sintered glass disks, can be varied within certain limits by partly filling the reservoirs with paper pulp or a similar substance.

The samples can be applied with a micropipette through one of the five small holes in the cover plate, which is provided with rims on all four sides so as to form a closed chamber saturated with vapour. The electrodes consist of two porous graphite blocks mounted in two separate chambers and provided with electrical connectors. Charring of the cellulose or excessive evaporation of the solvent does not occur. An increase in the flow-rate underneath the electrodes is prevented by the presence of twelve grooves perpendicular to the direction of the solvent flow. In some instances, it is advantageous to fill the electrode chambers with solvent.

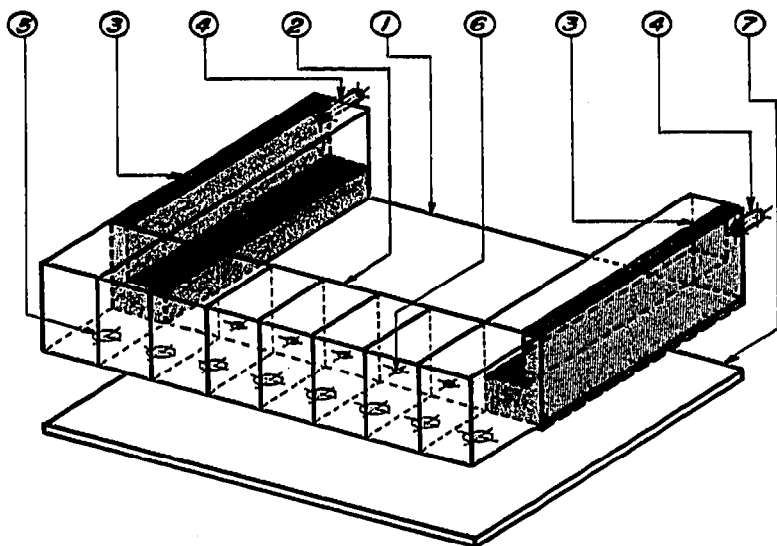


Fig. 1. Apparatus for simultaneous chromatography and electrophoresis on thin layers. 1 = 20×20 cm Perspex plate of 2 mm thickness; 2 = electrolyte reservoirs; 3 = graphite electrodes; 4 = electrical connectors; 5 = sintered glass disks of 11 mm diameter and 3 mm thickness; 6 = holes for sample application; 7 = glass plate or plastic sheet coated with cellulose layer; 3 mm of the layer material is scraped off on four sides.

RESULTS AND DISCUSSION

The technique was tested with mixtures of the aquochloro complexes of trivalent iridium, obtained by nuclear recoil or by aquation of labelled hexachloro complexes^{1,2}. A typical result is shown in Fig. 2. The identity of the constituents of the mixtures was determined from their behaviour in one-dimensional high-voltage or thin-layer electrophoresis (TLE) or thin-layer chromatography (TLC) and in two-dimensional consecutive separations, and was partly based on the applicability of Martin's additivity rule⁶. The number of separated spots is about half the number obtained when the analysis is performed in a consecutive manner, but is in perfect accord with the total number of peaks that can be detected, although not completely separated, by high-voltage paper electrophoresis^{1,2}. It can therefore be assumed that both the high-voltage electrophoretic and the "chromatophoretic" methods indicate the true number of radioactive products present in the sample solutions. The consecutive technique evidently results in the formation of a large number of artifacts, presumably by aquation of some of the aquohalo complexes during the intermediate drying process.

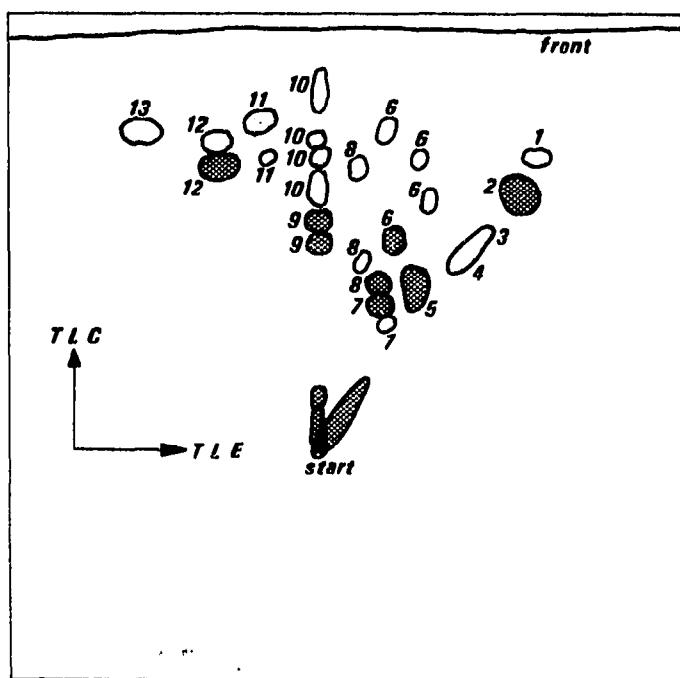


Fig. 2. Autoradiograph of a "chromatophoretic" separation of a mixture of ¹⁹²Ir-labelled aquohalo complexes of tetravalent and trivalent iridium. Layer: cellulose (Macherey & Nagel, MN 300), purified and coated on a 20 × 20 cm glass plate. Solvent/electrolyte: isopropanol(47.5 ml)-water(52.5 ml)-trichloroacetic acid (1 g) (pH = 4.0). Electrophoretic conditions: 600 V; 1 mA; 4 h; horizontal; 0°; qualitative detection with X-ray film. 1 = Ir₂Cl₁₀⁴⁻; 2 = IrCl₆²⁻; 3 = IrCl₅H₂O⁻; 4 = Ir₂Cl₉H₂O³⁻; 5 = IrCl₅H₂O²⁻; 6 = Ir₂Cl₈(H₂O)₂²⁻; 7 = IrCl₄(H₂O)₂⁻; 8 = Ir₂Cl₇(H₂O)₃⁻; 9 = IrCl₃(H₂O)₃; 10 = Ir₂Cl₆(H₂O)₄; 11 = Ir₂Cl₅(H₂O)₅⁺; 12 = IrCl₂(H₂O)₄⁺; 13 = IrCl(H₂O)₅²⁺.

From the results, the general conclusion can be drawn that inorganic ions can be subjected simultaneously to two principally different migratory forces. The tracks of some of the ions which can be followed visually are linear until they come too close to one of the electrodes. The ultimate positions of the spots are therefore the same as if the samples had been subjected to the two procedures in a consecutive mode. This was checked and confirmed by experiments using the apparatus shown in Fig. 1 by switching the voltage on after the solvent front had reached the edge of the plate. A limitation of the simultaneous technique is the requirement that the ions to be separated must show some electrophoretic mobility in the solvent that is normally used for their chromatographic separation. This limits the applicability to systems that can be chromatographed in solvents that contain water and dissolved electrolyte.

The method was also checked with mixtures of oxyphosphorus acids, the separations of which by various techniques have been studied in this laboratory⁷. A considerable reduction in time could also be achieved without a significant loss of resolution.

CONCLUSIONS

The simple apparatus described for the simultaneous application of thin-layer electrophoresis and thin-layer chromatography, although designed specifically for the separation of the aquochloro complexes of iridium, is likely to have a more general applicability. The major advantages of the method are a considerable reduction in the time required for the two-dimensional analysis and the possibility of analyzing compounds that cannot conveniently be separated by consecutive methods as a result of their instability or volatility. Comparison of the chromatograms after simultaneous and consecutive runs may therefore in some instances give qualitative information on the relative stability of some components.

An application of the technique seems to lie in the field of inorganic hot-atom chemistry, where one often deals with complex mixtures of relatively unstable products. Because of this instability and sometimes of the short half-life of the radioisotope used, fast separation methods are required.

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