

Quantitative Spot Test on Filter Paper and Examples of its Application

WHILE, so far, the spot test on filter paper, developed by Dr. F. Feigl and his co-workers, has been used exclusively for qualitative purposes, we have discovered a method for the estimation of various substances by using circular filter papers and the solvents which are commonly used in paper partition chromatography.

The procedure is as follows. At the centre of a circular filter paper a small drop of the solution of the sample containing a few milligrams of the substances under examination. After drying, solvent for partition chromatography is dropped in small portions (5-10 drops per min.) from a capillary tube on to the centre of the paper, thus causing development of the chromatogram. The substances contained in the sample mixture develop gradually to accurate concentric circles forming partition zones. For this manipulation a desiccator or Wit's filter bottle is used, in the bottom of which there are placed a small volume of partition solvent to saturate the air in the vessel with the vapour of the solvent used and a Petri dish containing the same solvent on which the filter paper (of about 11 cm. diameter) is placed. The development is complete when solvent has spread to a circle 7-8 cm. in diameter (about 30-40 min.). By using circular filter paper, we can work with much larger amounts of material in a shorter time than by the usual method of paper chromatography. Quantitative estimation is usually made by calculating the area of partition zones on the filter paper. It may, however, be effected, in general, more exactly by cutting up the paper, extracting and then applying colorimetry or densitometry by ultra-violet light to the extract. The chromatograms thus obtained are similar to those produced by Rutter's method². Our procedure was developed in the past few years independently of his report.

The following are a few examples of the application of quantitative spot tests on filter paper.

(1) *Estimation of methanol and ethanol.* In a preceding paper³, we reported a method for the detection of methanol, in which the potassium xanthogenates derived from a methanol-ethanol mixture by adding carbon disulphide and potassium hydroxide are submitted to paper partition chromatography using butanol saturated with 5 per cent sodium bicarbonate solution. Applying the technique outlined above, the ethyl xanthogenate is discernible as an outer circle, while that of methanol is located in an inner zone, as shown by ultra-violet light. The chromatogram is dried, cut up into its partition zones and the latter extracted by 5 per cent sodium bicarbonate. After sixty seconds from the addition of freshly prepared Grote's reagent (twice normal concentration) the estimation can be made electro-photometrically.

(2) *Estimation of R-acid and G-acid.* Both acids are apt to be produced in admixture as intermediates in the manufacture of dyes, and their separation was considered hitherto as a difficult problem. Applying our new technique to the development of the mixture of both acids (using butanol saturated with a 5 per cent solution of sodium bicarbonate) and following the process under ultra-violet light, the partition zones (outlined with pencil) are extracted with 5 per cent sodium bicarbonate solution. By addition of the diazonium salt of naphthionic acid,

there are produced red dyes, which can be estimated electrophotometrically.

Note added in proof. A somewhat similar technique of circular paper chromatography has been described recently by Giri and Rao⁴.

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¹ Feigl, F., "Qualitative Analyse mit Hilfe von Tüpfelreaktion".

² *Nature*, **161**, 435 (1948).

³ *Nature*, **163**, 511 (1951).

⁴ Giri, K. V., and Rao, N. A. N., *Nature*, **169**, 923 (1952).

Conductance in Non-aqueous Solutions

IN the extensive work of Fuoss, Kraus and co-workers¹ on the conductivity of solutions in media of widely divergent properties, these authors are led to the conclusion that, viscosity excepted, there is no specific influence due to the solvent, but that the dielectric constant of the medium alone determines conductivity data.

Since Fuoss and Kraus do not consider acid media, measurements of conductance of solutions in some organic acids are in progress in this laboratory to test the theory mentioned above. The following materials were used: 100 per cent glacial acetic acid (solvent-conductance $0.4-0.8 \times 10^{-8}$ mhos) obtained by fractionating 97 per cent acetic acid; 100 per cent propionic acid (solvent-conductance less than 3×10^{-10} mhos) obtained by fractionating a C.P. product; tetraethylammoniumpicrate (melting point 255.5°C .) prepared from silver picrate and tetraethylammoniumiodide. The conductance was measured with a Jones bridge²; a cathode-ray oscilloscope served as detector of current-minimum. A frequency of 1,000 c./s. was used. The conductance cells were designed according to Jones and Bollinger³, using non-platinized platinum electrodes. The measurements here described were made with a 125-c.c. cell (cell constant 0.00965). In preparing the solutions, all precautions were taken to prevent contact with the air.

Measurements were carried out at 25°C . with solutions of tetraethylammoniumpicrate in glacial acetic acid ($D = 6.25$) and propionic acid ($D = 3.15$) respectively, covering a wide range of concentrations. The accompanying graph shows the logarithm of the equivalent conductance (Λ) as a function of log molality (C). The dotted lines are from data of Fuoss and Kraus¹ on tetra α -soamylammoniumnitrate in mixtures of dioxane and water.

Although Fuoss and Kraus worked with a somewhat different salt (both salts, however, are rather strong electrolytes), it is obvious that our values fit in very well with theirs. So we may conclude that, also in the acid media, the dielectric constant is the determining factor for the conductance phenomena.

With the aid of the extrapolation method of Shedlowsky⁴ the following constants, corrected for solvent conductance, can be given for tetraethylammoniumpicrate in acetic acid: Λ_0 (equiv. conduct.