



Fig. 2. Current-voltage curve of a two-component system ( $10^{-4} M Pb^{++}$  and  $10^{-4} M Cd^{++}$  in  $0.1 M$  potassium chloride at  $c. 500$  r.p.m.)

in  $0.1 M$  potassium chloride) is shown in Fig. 2. The voltage-scanning rate was equal to that commonly used in conventional polarography.

Further details of this electrode, which appears to be a useful analytical tool, will be published elsewhere. Since this communication was written, our attention has been directed to recent work by Delahay *et al.*<sup>4</sup> in which he used a rotating electrode in voltameter studies of anodic stripping.

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<sup>1</sup> Gerischer, H., *Z. physik. Chem.*, **202**, 302 (1953).

<sup>2</sup> Berzins, T., and Delahay, P., *J. Amer. Chem. Soc.*, **77**, 6448 (1955).

<sup>3</sup> Ross, J. W., De Mars, E. D., and Shain, I., *Anal. Chem.*, **28**, 1768 (1956).

<sup>4</sup> Mamantov, G., Papoff, P., and Delahay, P., *J. Amer. Chem. Soc.*, **79**, 4034 (1957).

### Detection of Triterpenoid Acids on Paper Chromatograms

IN the course of an investigation of triterpene acids from natural sources it was necessary to devise tests for the detection of these compounds on alumina-impregnated filter paper.

A very convenient method is to spray the filter paper with a solution of phosphotungstic acid. Noller's reaction<sup>1</sup> has been used for the detection of triterpenoid compounds on filter paper; the Neher and Wettstein test<sup>2</sup> is also suitable for this. After spraying the paper chromatogram of triterpene acids with a solution of goat's blood<sup>3</sup>, haemolysis was observed.

The procedure is as follows. The paper chromatogram after development with the solvent is thoroughly dried by a current of hot air. It is next sprayed with a 25 per cent solution of alcoholic phosphotungstic acid and dried carefully for 2 min. in an oven kept at about  $115-118^{\circ}C$ . The paper then showed well-defined coloured spots against a white background;  $3-5 \mu m$ . of each component acid could be detected on the chromatogram.

The demonstration of triterpene acids on a paper chromatogram is also possible after treatment by the modified Noller reaction. Quantities of triterpene acids of about  $5 \mu m$ . on paper can be detected successfully after drawing the strip through a solution

Table 1. COLOURS OF SPOT TESTS FOR TRITERPENE ACIDS

Substance	Alcoholic phosphotungstic acid (25 per cent)	Stannic chloride in thionyl chloride (1 per cent)	Zinc chloride in benzoyl chloride
Poli-porenic acid A	Orange	Orange	Blue-purple
Glycyrrhetic acid	Yellow-brown	Yellow-brown	Yellow-brown
Ursolic acid I	Pink-purple	Pink→violet	Pink→violet→blue
Betulonic acid	Brown-violet	Yellow-violet	Yellow-brown
Oleanolic acid	Pink-purple	Pink→violet→blue	Pink→violet→blue

of 1 per cent anhydrous stannic chloride in pure thionyl chloride and heating it for 60–80 sec. at  $85-90^{\circ}C$ . Spots of different colours are obtained with the triterpene acids.

For the haemolysis test, a solution of defibrinated goat's blood in normal saline (1:8) is used. After spraying, the triterpene acids appeared as lighter spots on a tan background. In some cases, the spots become more distinct after the paper has dried and occasionally they were more readily discernible in transmitted light.

For the detection of triterpene acids on alumina-impregnated filter paper Gross's<sup>4</sup> and Belic's<sup>5</sup> reagents can be used. However, these reactions are not so useful in practice. Full details of this work will be published in *Chemia Analityczna* (Poland).

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<sup>1</sup> Noller, C. R., Smith, R. A., Harris, H. G., and Walker, J. W., *J. Amer. Chem. Soc.*, **64**, 3047 (1942).

<sup>2</sup> Neher, R., and Wettstein, A., *Helv. Chim. Acta*, **35**, 276 (1952).

<sup>3</sup> Fiedler, U., *Arzneimittel-Forsch.*, **4**, 213 (1954).

<sup>4</sup> Gross, D., *Nature*, **178**, 29 (1956).

<sup>5</sup> Belic, I., *Nature*, **178**, 538 (1956).

### Qualitative, Quantitative and Preparative Chromatography of Steroids on Fully Acetylated Paper

THE majority of methods developed for the paper chromatographic separation of steroids involve impregnation of the paper with a relatively polar, non-volatile liquid, such as propylene glycol, formamide or 'Phenylcellosolve', a less polar liquid being used as the mobile phase. Some 'reversed phase' techniques have also been applied, in which the paper is rendered hydrophobic by impregnation with 'Quilon', paraffin oil, kerosene or silicone and the mobile phase is a relatively polar liquid. Impregnation of the paper is usually difficult to carry out in a reproducible way and therefore  $R_F$  values may depend on slight experimental variations. Moreover, impregnation with a non-volatile liquid may interfere in quantitative determinations.

Chromatography on acetylated paper has the advantage of requiring no such impregnation. The separation of various substances on partially acetylated paper has been described by several authors<sup>1</sup>. It is, however, difficult to obtain a homogeneous