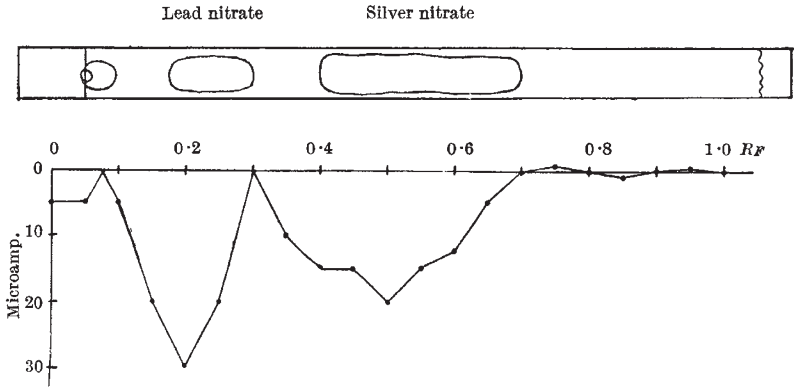

Solvent : butanol/pyridine/water, 100/20/20. Frequency : 15 Mc./s.

We have investigated the use of frequencies from 500 to 15,000 kc./s. and have found that the lower frequencies (500–5,000 kc./s.) promote greater sensitivity than the higher. We also find that each substance has an optimum frequency for maximum sensitivity. Further work is in progress in this direction.

The point of maximum concentration (required for calculation of R_F) is readily established (see diagram) by the apparatus even in the case of long spots caused by overlapping or tailing, and the sensitivity of the method is such that less than 1 γ of an inorganic compound can readily be detected. The sensitivity may be increased by the use of stronger currents.

It is essential that uniform filter paper should be used ; any quantitative filter paper will become almost electrically uniform after electrolysis, as previously described[1]. However, precise quantitative results will not be obtained until a filter paper with less background 'noise' is available. To this end, the solvents used in the papergram must leave no residue, since the smaller the background 'noise' the higher the sensitivity. The results obtained are as illustrated.

We have investigated the use of electric conductometry at mains frequencies, but the results were inferior to those obtained using high-frequency currents.

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Influence of Cations on the Ninhydrin Reaction for the Determination of Amino-Acids

THE micro-determination of α-amino-acids with ninhydrin in *n*-butanol is highly sensitive, but often inaccurate due to the retardation of colour formation and the production of various red or blue colours instead of the purple compound usually obtained. It was found that traces of cations had a marked influence on the speed of colour formation and its intensity and on the absorption spectra of the coloured compounds produced. (The effect of cations on the ninhydrin colour was employed for the preservation of chromatogram spots by Kawerau and Wieland[1].)

The addition of 0·1 ml. of a *M*/100 solution of any of the cations, Al^{3+}, Hg^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Sn^{2+}, Ag^{+} and Cu^{2+}, to a reaction mixture consisting of 10 μgm. alanine in 0·1 ml. water and 3 ml. of a 0·1 per cent solution of ninhydrin in *n*-butanol entirely abolished colour formation, which readily takes place when the reaction mixture is refluxed for ten minutes without the addition of cations. The presence of Ca^{2+}, Ba^{2+} and Cd^{2+} caused the formation of red colours with absorption maxima at 510 and 410 mμ (Ca^{2+} and Cd^{2+}) and 530 and 410 mμ (Ba^{2+}), which were spectrophotometrically detectable when about 1 μgm. of the cation was added to the reaction mixture. The absorption maximum of the normal colour at 570 mμ was progressively diminished and disappeared entirely when 20 μgm. of cation were present. The cation effect could be entirely eliminated by the addition of the chelating agent versene (the disodium salt of ethylene-tetraacetic acid). The coloured compound produced in its presence was uniform and had sharply defined absorption maxima at 570 and 410 mμ. The sensitivity of the reaction was considerably enhanced and exceeded that obtained by the addition of pyridine.

Good quantitative results could be obtained by employing the following method. A mixture, consisting of 0·1 ml. of the aqueous solution (containing 5–50 μgm. of the amino-acid to be determined), 0·3 ml. of a 0·3 per cent solution of versene in *M*/15 citrate buffer (*p*H = 5) and 3 mgm. ninhydrin dissolved in 3 ml. of aldehyde- and peroxide-free *n*-butanol, is refluxed for fifteen minutes in a 'Pyrex' reagent tube (20 × 250 mm.), using a cold finger condenser. After cooling, the mixture is diluted to 10 ml. with 50 per cent (v/v) ethanol and measured in any suitable photoelectric colorimeter at 570 mμ. The colour is stable for at least ten hours.

The method is easily adaptable for the evaluation of chromatogram spots. In this case, the spots should be located on the paper by one of the methods not employing the ninhydrin reaction[2]. The spots are cut out, the paper is cut into small pieces, added to the reaction mixture and treated as described above.

The method allowed the determination of the amino-acids glycine, valine, leucine, *iso*leucine, threonine, serine, lysine, arginine, histidine, glutamic acid, aspartic acid, tyrosine and methionine in the range of 0·3–4·0 gm. amino nitrogen, with an average accuracy of ± 3 per cent. The relation of optical density to concentration was linear over the same range.

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