

with low concentrations, which is not apparent unless the time of incubation on the gel is prolonged (18–24 hr.). With the exception of polygalacturonase and pectin-esterase, it has not been possible to separate enzymes, as each is resolved into two or more components and the components from different enzymes frequently overlap. The exact status of these components is as yet undetermined; for example, the number of components and the resulting pattern of the gel-developed chromatogram vary with amylases from different sources. In general, solvent-water mixtures are preferable to aqueous salt solutions as developing agents.

Mitchell, Gordon and Haskins³ found that both adenosine-deaminase and amylase could be resolved into components on a pile of filter paper disks with ammonium sulphate solutions as developing agents.

It is probable that the chromatographic technique is comparable to electrophoresis in that it separates enzyme proteins due to differences in physical properties rather than differences in enzymic behaviour, for example, kinetics. The R_F -value has been of little use in this technique, as it is markedly affected by slight changes in the conditions of development.

Full experimental details are being submitted for publication elsewhere.

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¹ Grl, K. V., *Nature*, **167**, 859 (1951).

² Reid, W. W., *Nature*, **166**, 569 (1950).

³ Mitchell, H. K., Gordon, M., and Haskins, F. A., *J. Biol. Chem.*, **180**, 1071 (1949).

In both laboratories the sodium tetrametaphosphate hydrolysate was prepared by heating 1 gm. of the tetrahydrate for 2 hr. at 70° C. with just sufficient alkali to break the ring. This was chromatographed alongside pure specimens of the orthophosphate, pyrophosphate, triphosphate, trimetaphosphate and tetrametaphosphate kindly supplied by Dr. B. Raistrick.

A number of solvent mixtures were used, of which *n*-propanol, water, 0.88 sp. gr. ammonium hydroxide (60, 20, 20 by volume respectively) is a good example. A strong spot which by colorimetric analysis accounted for 60 per cent of the hydrolysate appeared in a position where the tetraphosphate ion might logically be expected, and could not be ascribed to any of the other anions conceivably present.

During the course of this work, it was noted that a separation of orthophosphate, pyrophosphate and triphosphate anions could be obtained. Furthermore, it was observed that a sample of commercial sodium triphosphate, which by usual methods of analysis had a pyrophosphate content of 3–10 per cent, gave on chromatographic analysis two spots, the fainter one occupying the pyrophosphate position. It would seem, therefore, that a chromatographic separation of the phosphate anions should lead to an improved method for their quantitative analysis.

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¹ Raistrick, B., *J. Roy. Coll. Sci.*, **19**, 9 (1949).

² Thilo, E., and Rätz, R., *Z. anorg. Chem.*, **259**–**260**, 255 (1949).

³ Hanes, C. S., and Isherwood, F. A., *Nature*, **164**, 1107 (1949).

Chromatographic Evidence for the Tetraphosphate Ion

A KNOWLEDGE of the structure of phosphorus pentoxide, P_4O_{10} , enables predictions to be made concerning the mechanism of its hydration to orthophosphoric acid and particularly about the structures of the phosphoric acids formed intermediately¹. In order of their formation, these would be expected to be $H_2P_4O_{11}$, $H_4P_4O_{12}$ (tetrametaphosphoric acid), $H_6P_4O_{13}$ (tetraphosphoric acid) and eventually mixtures of $H_5P_3O_{10}$, $H_4P_2O_7$, and H_3PO_4 . Salts of tetrametaphosphoric acid have already been isolated and identified by X-ray and other methods from the alkaline hydrolysate of P_4O_{10} , and we have explored the use of paper chromatography as a means of separating the tetraphosphate ion. Aqueous solutions which would be expected to contain the latter were made by controlled alkaline hydrolysis of sodium tetrametaphosphate, using conditions analogous to those giving sodium triphosphate from sodium trimetaphosphate and similar to those described by Thilo and Rätz².

Chromatographic evidence for the existence of the tetraphosphate ion was obtained at Cambridge by one of the authors in association with Dr. C. S. Hanes using the techniques developed by Hanes and Isherwood³ in their study of phosphoric esters. This chromatographic evidence has been confirmed in detail by further work at Toronto.

Vapour Pressure of Isotopic Carbon Tetrachlorides

A THEORY of the vapour pressure relationship for isotopic molecules has been given by Topley and Eyring¹. It is, however, difficult to see *a priori* how to apply the theory in calculating vapour pressures for a particular case, in view of our lack of knowledge of the liquid state. The isotopic molecules of carbon tetrachloride would be expected to be very favourable cases for showing differences of vapour pressures if they exist. Grimm² carried out a multiple fractionation with a single small packed column and calculated that neighbouring isotopic molecules on the mass scale had boiling points not differing by more than 0.001° C. We have calculated from Grimm's results that his total fractionation was equivalent to 75 theoretical plates.

Modern packed columns have much higher efficiency and can be operated automatically. We have made use of three packed columns constructed by Dr. Newell Cook of the Chemistry Department of this College in an attempt to fractionate the isotopic molecules of carbon tetrachloride.

These columns have probable theoretical plate ratings of 100, 200 and 400 plates respectively, and are completely automatic in operation. We have operated the columns in such a manner that the first distillate was charged into the second column, etc. The reflux ratio used was 1,000 : 1. The last column was brought to equilibrium, and the specific gravity of the equilibrium mixture relative to the original