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THE PREPARATION OF ^{32}P LABELLED PHOSPHOROUS ACID

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ABSTRACT

Phosphorous acid labelled with ^{32}P has been prepared, on a small scale, starting from neutron-irradiated phosphorus. The compound is intended for tracer studies in the development of novel fungicides.

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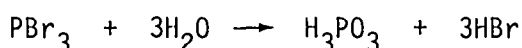
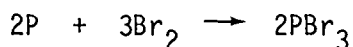
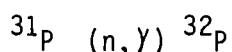
1. INTRODUCTION

Phosphorous acid has been shown to be effective in controlling fungal diseases in plants, even though it shows little direct anti-fungal activity. It has been suggested that the compound may stimulate the plants' own defence mechanisms [Guest 1986]. Research at the University of Melbourne has led to the development of new anti-fungal agents, based upon phosphorous acid. To study the mode of action of these compounds, and to trace their distribution within infected plant tissue, a radioactive form of phosphorous acid was required. The normal methods of synthesis have been assessed and modified to suit small scale radioactive preparation.

Phosphorous acid is most frequently prepared by the low temperature hydrolysis of a phosphorus tri-halide [Voigt and Gallais 1953]. The conversion of red phosphorus to the trihalide is readily achieved by a direct reaction of a stoichiometric quantity of the appropriate halogen with red phosphorus suspended in carbon tetrachloride [Gay and Maxson 1946]. The tribromide was selected for this preparation owing to the ease of measuring an exact amount of bromine, a liquid, as opposed to chlorine which is a gas. It is essential that the amount of halogen added does not exceed three mole equivalents, or penta halides will be formed, which, upon hydrolysis, will yield orthophosphoric acid. To prevent this unwanted side reaction, the amount of bromine added is 20 per cent less than the stoichiometric quantity.

2. EXPERIMENTAL

The sequence of reactions leading to pure ^{32}P labelled phosphorous acid may be summarised as below:



2.1 Irradiation of Phosphorus

Red phosphorus, which had been dried in vacuo over phosphorus pentoxide, was sealed in silica ampoules. Targets typically contained 100 mg of phosphorus and were irradiated in the AAEC's materials testing reactor HIFAR, at a neutron flux of 5×10^{13} neutrons $\text{cm}^{-2} \text{s}^{-1}$, for periods of up to seven days. Short-term irradiations produced approximately 40 MBq of ^{32}P per target per hour.

2.2 Preparation of ^{32}P Phosphorus Tribromide

The irradiated ampoule of phosphorus (100 mg) was placed in a short length of PVC tubing, and crushed using a screw-jack. The phosphorus was tipped into a 25 mL round bottom flask and suspended in carbon tetrachloride (7 mL). This suspension was brought to gentle reflux and treated with bromine (0.2 mL) in carbon tetrachloride (1 mL) over a period of five minutes. Reflux was continued until the solution was clear, usually after 15 minutes, when the solution was decanted into a clean 25 mL flask.

2.3 Preparation of Phosphorous Acid

The solution of phosphorus tribromide was cooled in an ice bath and stirred vigorously. Hydrolysis was accomplished by treating with water (1 mL) over a period of 30 minutes. It is essential that the solution be kept at a low temperature during hydrolysis to prevent disproportionation, which will result in the formation of ortho-phosphoric acid [Van Wazer 1958]. When hydrolysis was complete, the aqueous phase containing the ^{32}P phosphorous acid was separated from the carbon tetrachloride.

2.4 Purification

To separate the phosphorous acid from the hydrobromic acid, which is also produced during hydrolysis, the solution was made neutral with sodium hydroxide solution, then treated with excess barium chloride solution. The precipitated barium phosphite was filtered off, washed with water, and resuspended in water (10 mL). Treatment of the stirred suspension with cation exchange resin (Bio Rad AG 50W-X, 8H^+ form, 100-200 mesh) resulted in a clear solution of ^{32}P phosphorous acid, which was decanted from the resin beads and filtered.

2.5 Analytical

2.5.1 Identification

The material satisfied all of the requirements listed by Vogel [1957] under the heading 'Reactions of Phosphites', i.e. reduction of silver nitrate, permanganate and mercuric chloride, and the evolution of phosphine when treated with zinc and hydrochloric acid.

2.5.2 Radiochemical purity

Paper electrophoresis using 0.1 N lactic acid as electrolyte [Halmann and Kugel 1963] was found to give the most satisfactory separation of phosphorous acid from the most likely contaminant, ortho-phosphoric acid. Electrophoretograms were carried out at 8 V cm^{-1} over a period of three hours. Figure 1 shows a comparison of (a) the phosphorous acid, (b) ortho-phosphoric acid, and (c) a mixture of the two.

Phosphorous acid produced by the above method has been shown to be essentially free from contamination of orthophosphoric acid. Further proof of purity was obtained by titration with sodium hydroxide solution. Only two ionisable protons were noted, and treatment with barium chloride which will liberate the third proton of orthophosphate [Paris and Tardy 1946], failed to detect the presence of the most likely contaminant.

2.5.3 Quantitative estimation

The acid concentration is readily estimated by titration with standard sodium hydroxide solution. Two distinct end points are seen, at pH 4.5 and pH 9.0

3. DISCUSSION

Phosphorus-32 is readily detected by virtue of its β^- decay energy (1.71 MeV, 100 per cent). It is simple to estimate by liquid scintillation counting, and it is ideal for autoradiography. These properties make the nuclide quite useful in tracer studies, especially in biological systems.

The nuclide is normally prepared by the $^{32}\text{S} (n,p)^{32}\text{P}$ reaction, which yields "carrier free" material. The sequence of reactions in this preparation demands careful control of the stoichiometric amounts of reactants; this could not be practically managed with the minute mass involved in "carrier free" ^{32}P , so it was decided to use lower specific activity material produced by the $^{31}\text{P} (n,\gamma)^{32}\text{P}$ reaction. Although the neutron cross section of ^{31}P is low (0.2 barns), short irradiations produced adequate quantities of ^{32}P , approximately 40 MBq per target per hour.

All steps in the preparation are simple and readily adaptable to remote handling practice, and the entire preparation may be completed in three hours.

4. CONCLUSION

The method described provides a simple means of synthesising ^{32}P labelled phosphorous acid starting from reactor irradiated phosphorus. The material thus produced is expected to be of use to biological tracing.

5. ACKNOWLEDGEMENTS

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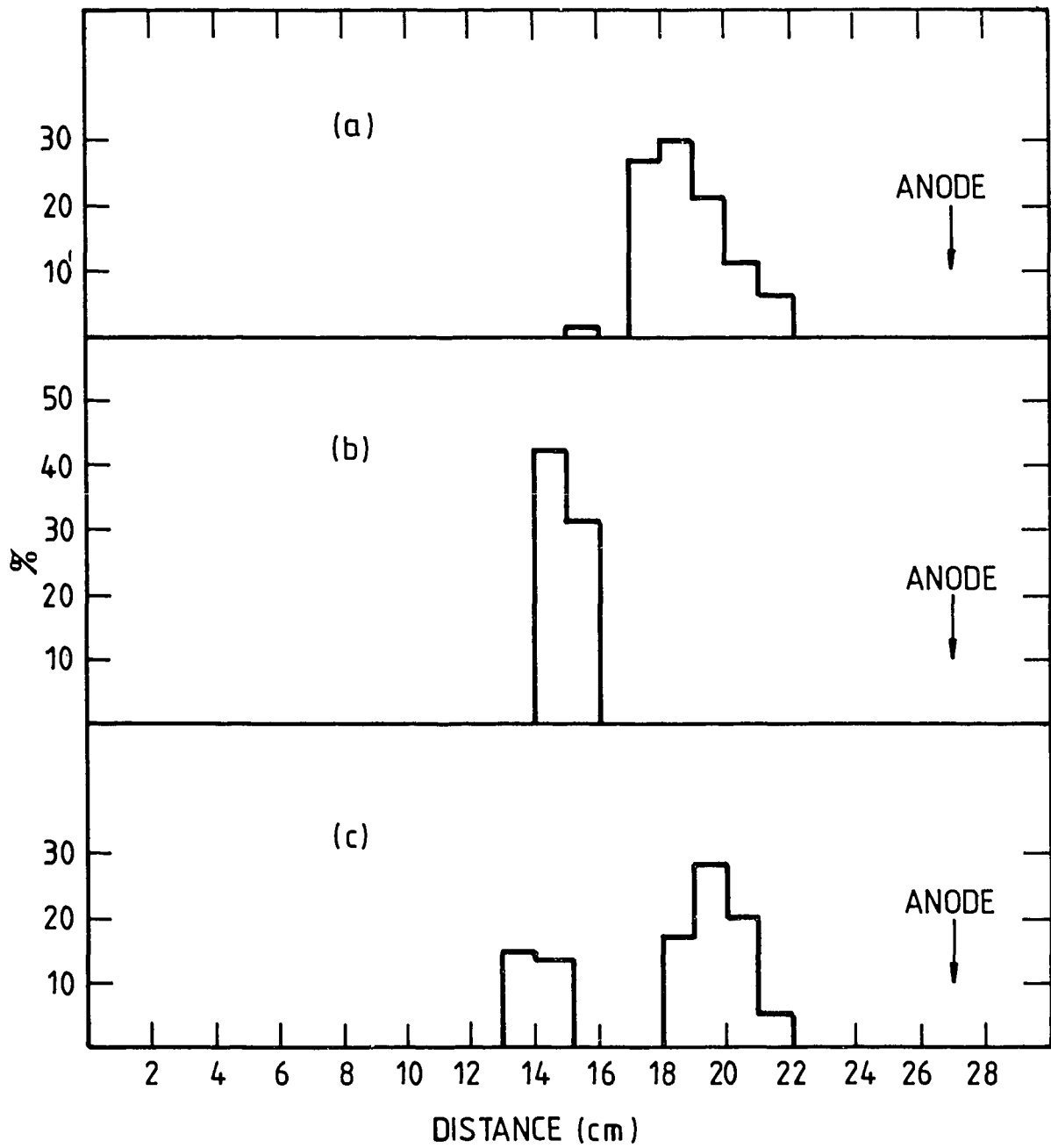


Figure 1 Electrophoresis of phosphorous acid