

PREPARATION OF PHOSPHORUS-33 LABELED POTASSIUM PEROXYDIPHOSPHATE, $K_4P_2O_8$

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SUMMARY

[^{33}P] Potassium peroxydiphosphate (PDP) was prepared by the electrochemical oxidation of [^{33}P] orthophosphate in alkaline solution. After purification by repeated crystallization from aqueous methanol, the radiochemical purity of the final product was 96.5% [^{33}P] PDP and 3.5% [^{33}P] orthophosphate. Overall radiochemical yield was 81%; electrochemical yield was 40%.

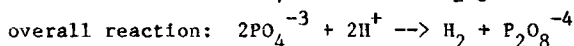
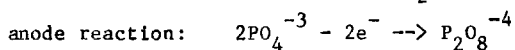
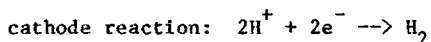
KEY WORDS: Potassium peroxydiphosphate, [^{33}P] phosphorus, preparation, electrochemical, chromatography.

INTRODUCTION

PDP is of interest as an oral health agent. (1) To provide material for radiotracer experiments [^{33}P] PDP was prepared and purified.

PDP is a highly water soluble crystalline solid with good chemical stability in neutral or alkaline solution. (2) It was first prepared in 1910 by a chemical method. Later work showed that a better product resulted from the electrochemical oxidation of alkaline solutions of orthophosphate in the presence of certain catalysts. Fluoride ion is the preferred catalyst because of greater ease of purification. (3, 4)

The electrochemical oxidation of orthophosphate proceeds as follows:



The anolyte and catholyte solutions are kept separate by a porous membrane, which in this case, was an alundum thimble. As the reaction proceeds, the anolyte pH falls because potassium ions migrate to the cathode. In order to maintain anolyte K^+ level and pH, concentrated KOH solution is metered into the anolyte.

^{33}P was employed as the radioactive label, instead of the more common ^{32}P because the former has a longer half life (25.3 days vs. 14.3 days for ^{32}P).

REAGENTS AND APPARATUS

The anolyte was an aqueous solution of 8.0 molar potassium hydroxide, KOH, 2.0 molar orthophosphoric acid, H_3PO_4 , and 2.4 molar hydrofluoric acid, HF (anolyte density = 1.45 g/ml). 50% aqueous KOH was used to maintain anolyte pH. [^{33}P] H_3PO_4 was purchased from New England Nuclear, Boston, MA (#NEZ 080, carrier free). The catholyte was a 1.19 molar KOH and 0.96 molar H_3PO_4 solution in water.

The anolyte was contained in a flat-bottomed alundum thimble (#E9890, 12 x 54 mm, SGA Company, New Jersey) which rested upright in a 400 ml plastic beaker containing the catholyte. The anode was a spiral coil platinum wire (0.064 inches, diam.) while the cathode was a coil of 1/8 inch #304 stainless steel tubing wound loosely around the alundum thimble. Cooling water flowed through cathode tubing during the reaction.

Regulated constant current was used for the electrochemical reaction. Full wave rectified DC from a 12V automobile battery charger was first filtered to essentially smooth DC by a 9000 uF capacitor. The filtered DC was regulated at 0.36A using an integrated circuit regulator (#LM 338K, National Semiconductor Corp., Santa Clara, CA) wired in the current-regulating mode. At that current level, the reaction was completed in about 2.5 hours. The applied potential fluctuated between 3.7 and 3.9 V. Anode current density was $0.05 A/cm^2$.

Thin layer chromatography and visualization were carried out by a modified method of Clesceri (5) on cellulose plates (#8011, Type MN-300, Analtech, Newark, DE) which were developed using the solvent 82.5 ml dioxane + 17.5 ml

water + 5.0 g trichloroacetic acid + 0.25 ml ammonia. This system can separate peroxydiphosphate (Rf 0.17) from orthophosphate (Rf 0.87) and pyrophosphate (Rf 0.33). Spots were disclosed by both autoradiography (ARG) and spraying with ammonium molybdate and stannous chloride solutions. Spots identified by ARG were scraped into liquid scintillation vials, shaken with 1 ml water plus 15 ml LSC cocktail (Biofluor, New England Nuclear) and counted. All LSC counting was done in a Beckman #LS 6800 counter.

A strong anion-exchange resin (AG-1-X8, Bio-Rad Labs., Richmond, CA) system was used as an additional radiochemical purity check. With this resin, orthophosphate is eluted first by 0.25 molar KCl in 1.0 molar, pH 5.2 acetate buffer, while PDP remains on the column. PDP is eluted next with 2 molar KCl in the acetate buffer. Aliquots from the two fractions were counted as above. The two methods for radiochemical purity agreed very closely for all samples tested.

Chemical purity was quantitated by a standard cerometric titration using 0.01 molar reagents. (6) Fluoride was measured with specific ion electrode following standard procedures.

EXPERIMENTAL AND RESULTS

0.1 ml of cold carrier 1% K_2HPO_4 was added to 20 mCi of $[^{33}P]H_3PO_4$ as received. After sampling, the vial contents were evaporated to near dryness on a steam bath with a gentle stream of nitrogen. The vial was then rinsed out with several portions of anolyte solution until the total anolyte weight was 10.6 g. After removing small samples for counting, 10.35 g anolyte (containing 7.13 mmol P) was available in the anode compartment for the reaction. As the anolyte was added to the thimble, catholyte was added simultaneously to the surrounding beaker, keeping the liquid levels in both compartments at the same height.

After starting flow of electric current, 50 μ l samples were removed every 0.5 hours and titrated to follow the reaction course. When successive titrations indicated no additional formation of PDP, the reaction was halted. The electrolysis time was about 3 hours.

To purify the PDP, the anolyte was transferred to a 30 ml centrifuge tube where it was concentrated to about twice the calculated PDP weight on a steam bath under a stream of N_2 . The resulting slurry was centrifuged, the clear supernatant removed, sampled and discarded. Just enough water was added to dissolve the remaining cake in the centrifuge tube. The PDP was then precipitated out by adding twice the solution volume of absolute methanol. The resulting precipitate was filtered, washed with methanol and dried at $50^\circ C$. The methanol precipitation and drying was done three times.

The gravimetric yield of purified product was 2.33 g containing 91.7% PDP (6.17 mmol PDP). The radiochemical purity was 96.5% [^{33}P] PDP and 3.5% [^{33}P] orthophosphate which was the only impurity detected. Overall radiochemical yield was 81%. About 10% of the starting radioactivity was found in the catholyte. Total radioactive recovery was close to 100%, which included product, supernatants, filtrates, and catholyte. Fluoride in the product was 0.01% F^- . The specific activity was 2.4 mCi/mmol (88MBq/mmol). The electrochemical yield was 40%. The principal electrochemical by-product appeared to be ozone, as judged by potassium iodide-starch test paper and odor.

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