

Preparation and Analysis of Cyclotri- and Cyclotetraphosphate and Their Hydrolysis Products in Soil

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Cyclotriphosphate ($\text{Na}_3\text{P}_3\text{O}_9$) and cyclotetraphosphate ($\text{Na}_4\text{P}_4\text{O}_{12}$) are not strongly sorbed by soil constituents. Potential movement and efficient plant utilization of P from these compounds are dependent on the hydrolysis of the cyclophosphate ring structure and their hydrolysis products. The objectives of this study were to prepare pure useable quantities of these cyclophosphates and their hydrolysis products and to extract, separate, and analyze these compounds after application to diverse soils. Cyclophosphates of high purity (>99.0%) were prepared, and improved methods of extraction and analysis by ion chromatography were developed. Cyclophosphates and their hydrolysis products were extracted from soil using a sequential water/0.5 M H_2SO_4 /1.0 M NaOH extraction that maximized P recovery and minimized hydrolysis of cyclic and linear phosphates during the extraction procedure. Gradient elution chromatography separated cyclic phosphates and their hydrolysis products. Separation and direct quantitative analysis of the applied cyclophosphates and their hydrolysis products were accomplished in <15 min.

KEYWORDS: Cyclophosphates; ion chromatography; hydrolysis; P sorption

INTRODUCTION

Cyclophosphates [rules for chemical nomenclature are used to designate cyclophosphates (*I*)] are not substantially sorbed to soil constituents. It is hypothesized that the mobility and efficiency of phosphorus (P) utilization by plants could be enhanced through the use of these compounds. Cyclotriphosphate ($\text{Na}_3\text{P}_3\text{O}_9$; C3P) and cyclotetraphosphate ($\text{Na}_4\text{P}_4\text{O}_{12}$; C4P) of >99% purity are not commercially available. Pure cyclophosphates have not been produced on a commercial scale for use as fertilizers. However, if a distinct advantage of these compounds in agriculture over currently used phosphate compounds can be established, stable cyclophosphate-containing fertilizers could be produced for field application. The intent would be to produce phosphate fertilizers with a significant proportion of the phosphate as cyclotri- and cyclotetraphosphate rather than extremely pure cyclophosphates for commercial use. Prior to detailed studies of hydrolysis rates in soil and the relative efficiency of these compounds to plants, effective methods of preparation, extraction, and analysis of cyclotri- and cyclotetraphosphate and their hydrolysis products must be developed. There has been little research reported on the extraction and analysis of cyclophosphates and their hydrolysis products in soil. Lucci (2) and Blanchar and Hossner (3) first reported that simple C3P was not appreciably sorbed by soil. Lucci (2) studied the behavior of C3P and C4P in samples of

kaolin as well as three soils from Italy. This study showed that substantially less cycloP (cyclotri- and cyclotetraphosphate) was fixed compared to orthophosphate (monoP). Blanchar and Hossner (4) reported no significant sorption of C3P in an Elliot soil, whereas there was substantial sorption of monoP and short-chain polyphosphate (tri- and diphosphate). Further studies reported that C3P remained water soluble in midwestern U.S. soils (4, 5). Rates of hydrolysis of C3P in soils were rapid with a half-life of 5 h in an Elliot soil. Quantifiable amounts of C3P were found in only 2 of 32 soils after 24 h. The study of C3P was expanded by Busman and Tabatabai (5–7) to examine the behavior of C3P in different soil types and the chemical and biological factors that contribute to C3P hydrolysis. Dick and Tabatabai (8) reported that ~30–50% of applied C3P persisted in soils beyond 14 days. Their method, however, relied on the determination of monoP as a measure of C3P hydrolysis and relatively high rates of P application (0.500 g of P kg^{-1} of soil). No monoP is produced upon initial hydrolysis of the cyclophosphate ring structure; therefore, evaluation of C3P hydrolysis in soil cannot be accurately determined by measuring the release of monoP.

Solution chemistry indicates that larger cyclophosphates such as C4P may be more stable than C3P (9, 10). General factors governing the hydrolysis of cyclo and linear phosphates include temperature, pH, enzymes, colloidal gels, complexing cations, and ionic environment in solution (8, 11–15). Sodification had little effect on hydrolysis at an exchangeable sodium percentage (ESP) of <32.8, whereas at an ESP of 48.0, the rate of hydrolysis of condensed phosphates was retarded (16). Kura

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Table 1. Selected Chemical and Physical Properties of Soils

soil series	pH	texture			CaCO ₃ (%)	organic C (%)	dithionite extractable		cation-exchange capacity (cmol _c kg ⁻¹)
		sand (%)	silt (%)	clay (%)			Al (g kg ⁻¹)	Fe (g kg ⁻¹)	
Branyon	7.40	13.1	30.6	56.4	2.04	0.99	1.08	4.01	38.8
Crockett	6.24	63.4	28.0	8.6		0.71	0.26	2.61	6.6
Wilson	5.54	37.7	39.2	23.1		0.90	0.78	2.94	12.3
Falba	4.78	69.7	22.6	7.6		0.45	0.39	1.11	4.3

and Tsukuda (17) found that the hydrolysis rate of cyclic phosphates (C3P and C4P) was influenced by the degree of complex formation with cations. The hydrolysis rate was most strongly affected by Al³⁺. Crowther and Westman (18) and Kura (19) reported that C4P is more resistant to hydrolysis than C3P when in solution. The characteristics of greater resistance to hydrolysis may combine to provide greater plant availability of C4P over C3P and linear polyphosphates. Watanabe et al. (9) reported that the activation energy for the hydrolysis of both C3P and C4P increased in acidic solutions and the log *A* (frequency factor) for the hydrolysis of both cyclophosphates decreased with an increase in the pH value. Both the rate constant and the activation energy for cyclophosphates increased as the pH was increased in alkaline solutions.

There is a need to develop procedures to extract cyclophosphates and their reaction products from soils without additional hydrolysis due to the extracting solution and to quantify their concentrations in the extracting solution. Much of the research has utilized simple linear polyP and depended on the analysis of monoP in the extraction solution to measure rate constants. Recovery of applied cycloP and the associated reaction products has not been reported in many of the studies.

The objectives of this study were to (1) prepare relatively pure (>99% purity) C3P and C4P, (2) improve the recovery of C3P and C4P and their hydrolysis products from soils, and (3) analyze C3P and C4P and their hydrolysis products in the extraction solution by ion chromatography.

MATERIALS AND METHODS

Soils. Four soils were chosen to represent a range of pH, texture, and active Al and Fe (Table 1). These properties, along with biological activity, have been shown to affect the hydrolysis of condensed phosphates [soils were classified as fine, smectitic, thermic, Udic Haplustert (Branyon clay); fine, smectitic, thermic, Udertic Paleustalf (Crockett sandy loam); fine, smectitic, thermic, Typic Aquic Paleustalf (Falba sandy loam); and fine, smectitic, thermic, Oxyaquic Vertic Haplustalf (Wilson silt loam)].

Surface soils (0–15 cm) were sampled in December and January, air-dried, sieved through a 2-mm screen, and then stored at 3 °C for further use. Soil pH was measured with a combination glass electrode after 10 g of soil had been shaken with 25 mL of deionized water for 15 min. Percent CaCO₃ was determined according to the method of Bundy and Bremner (20) and organic C according to the method of Nelson and Sommers (21). Dithionite-extractable Al and Fe were quantified as outlined by Jackson (22). Cation-exchange capacity (CEC) was determined using the method of Sumner and Miller (23). Soil particle fractionation was evaluated by using the hydrometer method (24). Moisture contents at 0.033 and 0.10 MPa tension were determined by the use of pressure plates (25).

Preparation of Cyclophosphates. Simple structures and chemical formulas for the compounds used in this study are presented in Figure 1.

Cyclotriphosphate. A modification of the general purification procedure of Greenfield and Clift (26) was followed. Fifty grams of sodium C3P (anhydrous, 95–97%, Sigma Chemical Co., St. Louis, MO) was dissolved in a 500-mL glass beaker with 200 mL of deionized water at 70 °C. One cubic centimeter of diatomaceous earth was added

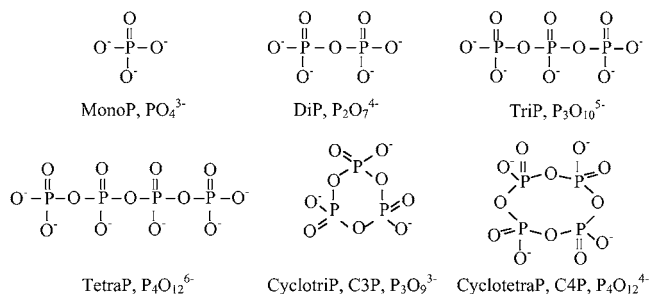


Figure 1. Simple representation of cyclotri- and cyclotetraphosphate and their hydrolysis products.

to the dissolved P with stirring to sorb any linear phosphates. The mixture was vacuum filtered through a 9-cm Büchner funnel using a medium fast quantitative filter paper. The filtrate, which contains the C3P, was clear. Upon cooling to near 30 °C, 80 mL of 95% ethanol was slowly added dropwise from a buret over 15 min. White gelatinous material began to form at ~50 mL of ethanol addition. The beaker was set aside at room temperature (22 °C) upon completion of ethanol addition for 36 h, during which time much of the fluffy, gelatinous material precipitated. The clear solution above the precipitate was decanted after 36 h, and the remaining mixture was filtered and washed briefly with ~10 mL of cold 50% ethanol. The material was freeze-dried for 36 h, ground in a mortar, and stored at 3 °C.

Cyclotetraphosphate. An ice bath was prepared in a 10-L plastic wash basin placed on a magnetic stirrer. Livestock grade salt (several hundred grams) was mixed with the ice in the wash basin. A 600-mL glass beaker (reaction vessel) was set as deeply as possible in the center of the ice so that the beaker bottom was close to or on the bottom of the basin. Temperatures around the outside of the beaker were measured at -11 to -9 °C. Ice-cold salt water was added to a depth of 5 cm in the plastic basin to maximize the cooling contact with the glass beaker.

A modified procedure outlined in Greenfield and Clift (26) with suggestions of other workers (E. J. Griffith, Monsanto Co., St. Louis, MO) was used to produce pure C4P. Quantities suggested in Greenfield and Clift (26) were divided by 5 for this preparation.

Four grams of analytical grade NaHCO₃ was dissolved in 200 mL of deionized water at room temperature and the solution cooled overnight in a refrigerator at 3 °C. This solution was placed in the reaction vessel and was further cooled to -2 °C. The NaHCO₃ solution was rapidly stirred with a 5-cm stirring bar on a magnetic stirrer. A combination glass pH electrode and a thermometer were placed in the reaction vessel. One hundred and fifty grams of analytical grade P₄O₁₀ (Fisher Scientific Co., Pittsburgh, PA; >99% purity) was slowly sprinkled directly into the solution in the reaction vessel. It was determined that mixing the P₄O₁₀ with dry CO₂ was not necessary, nor was it essential that the hygroscopic material be laboriously protected from sorption of water vapor. The temperature of the solution in the reaction vessel was not allowed to exceed 3 °C as the P₄O₁₀ was added, a temperature that was substantially below that reported in previous work (27, 28). The conversion of P₄O₁₀ to C4P was greatly improved, and the production of other linear phosphates, which are more likely to form at the high microsite temperatures, was minimized. During the evolution of CO₂ from the reaction vessel, drops of isoamyl alcohol were added to cut the froth and ensure that the P₄O₁₀ went directly into the bicarbonate solution. When the pH of the solution dropped below 8.5, a small amount of solid NaHCO₃ was added to control the solution pH. The speed of NaHCO₃ addition was regulated so that the

pH of the solution did not exceed 8.5 (a total of 144 g of NaHCO_3 , including the initial 4 g, was required). Up to 23 h was needed to neutralize the bicarbonate with P_4O_{10} to a pH of 4.5 without exceeding the temperature limit. The total P_4O_{10} used was near 110 g. About 30 mL of deionized water was added to the reaction vessel after 1 h to facilitate stirring of the increasingly viscous solution. The suspension was transferred to a Büchner funnel and vacuum filtered. Preliminary analysis indicated 80–90% C4P in the crystals on the filter paper and <50% in the filtrate that was discarded.

The impure C4P crystals were dissolved nearly to completion in ~600 mL of deionized water (a small amount of sticky material proved to be insoluble) along with 10 cm³ of diatomaceous earth. The suspension was stirred and then filtered as before to remove insoluble materials and any long-chain P sorbed on the filter agent. Ethanol was slowly added from a buret (2–4 mL min⁻¹) to the clear filtrate with stirring until a snowy precipitate quickly appeared. At this point, the solution was ~27% ethanol by volume. The solution was allowed to stir for 10 min. Crystals were again filtered and washed with 50% ethanol from a wash bottle. Initial ion chromatography (IC) analysis detected small amounts of hydrolysis products, so a second crystallization was necessary and the above steps were repeated. The pure C4P was freeze-dried and stored at 3 °C.

X-ray analysis was conducted on powdered samples with a Philips PW 1316/90 wide-range goniometer employing Cu K α radiation, a theta compensating slit, and a graphite monochromator at 500 counts s⁻¹. Continuous scans were obtained from 2 to 36 degrees 2 theta ($^{\circ}2\theta$).

Samples of each P compound were dissolved in water and analyzed for elemental composition. MonoP was determined in the presence of polyP according to the method of Dick and Tabatabai (29). Polyphosphate was determined by acidifying and hydrolyzing to monoP on a hotplate (6). After correction for monoP, a theoretical formula weight was calculated for each P compound. Anhydrous formula weights were then compared to determine the moles of water of hydration for each compound.

Extraction of Phosphates from Soil. Soils (3 g) were weighed into liquid scintillation vials. All phosphate compounds were applied in 0.3 mL of solution to provide 0.100 or 0.400 g of P kg⁻¹ of soil. The P solution was applied uniformly over the surface of the soil and mixed followed by addition of water to bring the soil to 0.033 MPa water content. The screw cap was removed and the vial top covered with Parafilm that was provided with a pinhole large enough to allow gas exchange but restrict drying of the sample. Samples were weighed on a daily basis, and moisture content was adjusted by adding fractional milliliters of deionized water. Soil samples were incubated from 0 to 552 h prior to extraction of the added P.

Scintillation vials with the P-treated soils received 8 mL of water, were capped, and were placed on a vortex mixer for 1 min to disperse the particles. Soil samples were spread uniformly on the filter paper that had been vacuum-sealed in a Büchner funnel and leached with 8 mL of water in 2-mL increments. Preliminary extraction of Branyon c and Wilson sil soils was extremely slow (20–30 min for Branyon c). To speed extraction, 1.0 and 0.2 g of diatomaceous earth (Sigma Chemical Co.) were mixed with the Branyon c and Wilson sil soils, respectively. Leaching time was decreased to 5–8 min. The water extract was collected in a separate 40-mL glass tube placed inside a 1-L vacuum flask that was removed when water leaching was complete.

Extraction was continued by leaching with eight 2-mL increments of 0.5 M H_2SO_4 followed by eight 2-mL increments of 1.0 M NaOH. The acid–base leachate was mixed with the water extract and the pH immediately adjusted to ~2.0 with either HCl or NaOH. The solution was brought to volume in a 50-mL volumetric flask for samples receiving 0.100 g of P kg⁻¹ and in a 100-mL flask for samples that received 0.400 g of P kg⁻¹. A portion of the sample was poured into a beaker, mixed with 0.1 g of activated carbon per 10 mL of solution to sorb soil organic matter, and filtered using a 10-mL syringe and 25-mm microfilter holder with a 0.22- μm (pore size) acetate filter prior to analysis by ion chromatography.

Analysis by Ion Chromatography. Phosphate species were separated using a Dionex model 4000i ion chromatograph (30) equipped with a gradient pump. The unit used in this work contained the following components: (1) four eluent reservoirs (1 and 2 L), each

Table 2. Results of Preparation, Compositional Analysis, and Working Formula Weights of Polyphosphates (Means of Five Separate Analyses)

compound	purity (%)	phosphate		formula wt (g mol ⁻¹ mol)	water (%)	CV ^a
		mono (%)	di (%)			
$\text{Na}_4\text{P}_2\text{O}_7$ diP	99.8	≤0.2	99.8	272.5	0.4	5.0
$\text{Na}_5\text{P}_3\text{O}_{10}$ ^b triP	98.9	0.8	0.2	488.4	6.7	5.1
$\text{Na}_3\text{P}_3\text{O}_9$ ^c C3P	99.7	≤0.1	≤0.1	331.6	1.4	5.1
$\text{Na}_4\text{P}_4\text{O}_{12}$ C4P	99.1	0.7	≤0.1	428.0	1.1	6.1

^a Coefficient of variation. ^b Commercial source was 89.7% triphosphate and purified as outlined by Greenfield and Clift (26). ^c Commercial source of cyclotriphosphate was 97.3% pure.

with a He gas supply for degassing of eluents; (2) a dual-piston pump; (3) an eluent manifold capable of gradient mixing of eluents; (4) a sample injection device equipped with a 16- μL injection loop; (5) an organic matter guard column (NG-1) and a general guard column (AG-5A) to protect the separator column by removing particulate matter and other potentially damaging substances from the eluent stream; (6) separator columns [two AS-5A columns in succession, each containing 20 mm.(+) of weak polystyrene divinylbenzene anion-exchange resin] to effect conventional ion-exchange separation; (7) a postcolumn membrane-exchange site (anion micromembrane suppressor, AMMS) where species of interest were converted to their highly conductive strong acids; (8) a supply of regenerant to the membrane-exchange site, 9) an N₂ gas supply for operation of valves and pressurization of the regenerant reservoir; (10) a conductivity detector with a temperature correction microprocessor; and (11) a plotter and integrator (Spectra-Physics model 4290, San Jose, CA). Individual P compounds were analyzed with an isocratic run at 0.900 M NaOH to determine retention time. The IC conditions were optimized at a flow rate of 1.5 mL min⁻¹ to separate all P from SO_4^{2-} in the chromatogram. Optimization was obtained by using a smaller injection loop (16 μL) and programming the IC to equilibrate with 0.024 M NaOH. The P sample in the injection loop was injected at 0.1 min (chromatograph time) into an eluent stream of 0.024 M NaOH. The initial concentration was programmed to increase linearly to 0.100 M NaOH at 6.0 min using a gradient procedure. Eluent flow at 0.100 M NaOH continued until ~1 min before elution of the final P species (9.0–11.0 min depending on the sample components) when the gradient program reverted to time zero and began pumping 0.024 M NaOH again. Baseline shift was experienced due to the use of gradient elution chromatography. Both peak integration and peak height were acceptable methods for evaluating phosphate species concentration but, using standard samples, it was determined that peak height provided more accurate data. Baselines were drawn by hand for each peak, and then the peak height at midpeak was measured to the nearest 0.5 mm.

RESULTS AND DISCUSSION

Purity of Cyclophosphate Compounds. Preparation and purification methods were successful in preparing useful, high-purity P compounds for soil application and standard analysis. The compositional analysis and working formula weights of the P compounds used for standards and soil application are presented in **Table 2**. Commercial diP was purchased that was 99.8% pure. Triphosphate was purchased that was 87.9% pure and recrystallized to obtain the 98.9% pure material (26). The sodium C3P and C4P compounds were prepared to 99.7 and 99.1% purities, respectively. Commercial C3P could be purchased that was 97.3% pure. The amounts of monoP, diP, water of hydration, and working formula weight for each of the materials were determined (**Table 2**). X-ray diffraction was used as a secondary proof to confirm the composition of the triP, C3P, and C4P. The diffractogram for C3P (**Figure 2**) was compared to that reported by Corebridge and Tromans (31). The 1.04 nm peak is reduced in size, and it disappeared for the

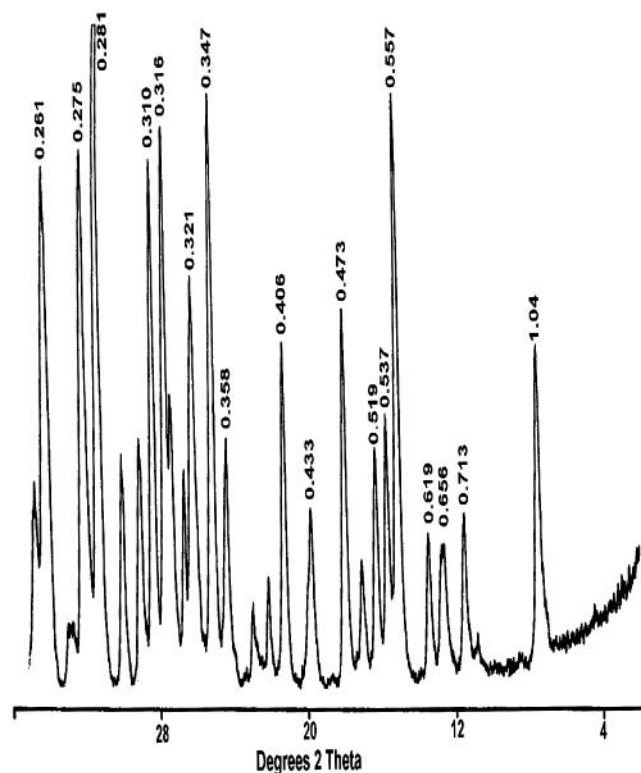


Figure 2. Random powder diffractogram of air-dried sodium cyclotriphosphate (C3P) prepared by single reprecipitation of commercial reagent by ethanol addition. Spacing values are in nanometers.

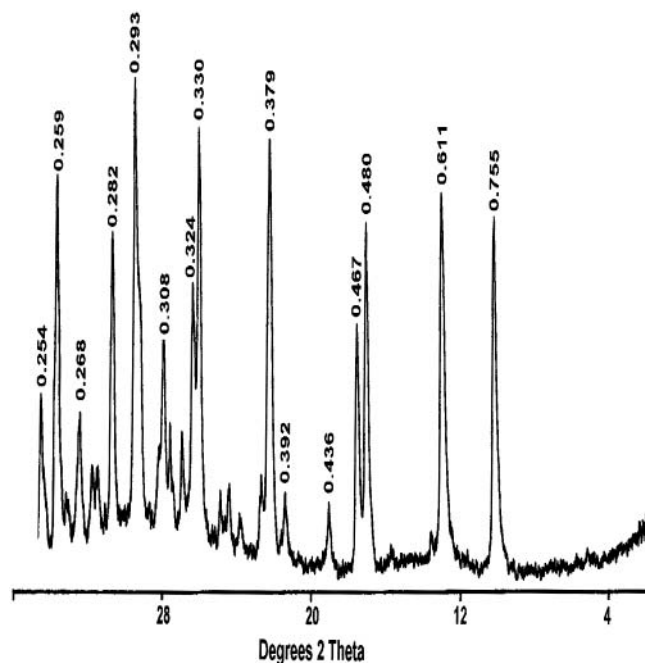


Figure 3. Random powder diffractogram of sodium cyclotetraphosphate (C4P) prepared by double reprecipitation of the hydrolysis product of P_4O_{10} and water in $NaHCO_3$ by ethanol addition. Spacing values are in nanometers.

anhydrous compound; otherwise, the similarity was excellent. A diffractogram for C4P (Figure 3) was strongly correlated with patterns found in Corebridge and Tromans (31) and the Mineral Powder Diffraction File Data Book (32). The sample was less hydrated than the sample reported by Bell et al. (27). Experience has indicated that higher quality precipitation products are acquired through slow, cautious addition of ethanol. Time must

Table 3. Recovery of Soil-Applied Cyclic Phosphate Following Incubation with Four Soils from 0 to 552 h

soil series	no. of samples	P source	rate of application (g of P kg ⁻¹)	mean recovery of soil-applied P ^a (%)
Branyon	18	C3P	0.100	47.4 ± 12.1
Branyon	42	C3P	0.400	87.0 ± 7.0
Branyon	24	C4P	0.100	81.8 ± 7.5
Branyon	63	C4P	0.400	84.0 ± 9.0
Crockett	24	C3P	0.100	92.2 ± 4.09
Crockett	24	C4P	0.100	92.6 ± 5.9
Wilson	30	C3P	0.100	93.4 ± 4.5
Wilson	24	C4P	0.100	89.7 ± 3.8
Falba	144	C3P	0.100	97.8 ± 4.6
Falba	81	C3P	0.400	97.9 ± 3.9
Falba	144	C4P	0.100	98.1 ± 3.8
Falba	90	C4P	0.400	98.9 ± 2.3

^a Mean ± one standard deviation. Data represent recovery across the entire incubation period.

be allowed for the crystals to form. Poor and incomplete precipitation occurred if ethanol was added too quickly. Following the final filtration, P samples prepared at room temperature were prone to hydrolysis. Heating of samples was avoided as efforts to speed the drying process by applying heat also resulted in hydrolysis. The use of vacuum desiccation and freeze-drying helped preserve samples both during and after drying.

Extraction of Soil Applied CycloP. The extraction procedure was the result of considerable testing of the recovery of soil-applied P and the potential and real effects of the extraction procedure on hydrolysis of cycloP. Initial tests indicated that very little hydrolyzed P (monoP, diP, triP, and tetraP) was recovered in the water extract. It was noted, however, that when a water pre-extraction was not used, hydrolysis of C3P was substantial (~15%) during application and immediate extraction using 0.5 M H_2SO_4 followed by 1.0 M NaOH. If NaOH was omitted from the procedure, C3P hydrolysis was 10.5%. Cyclotetraphosphate hydrolysis under identical circumstances was much less, totaling ~2–4%.

Recovery of applied P is a concern for the determination of cycloP hydrolysis in soils. Data in Table 3 summarize the recovery of soil-applied P using the sequential extraction procedure (water/ H_2SO_4 /NaOH) after application of C3P and C4P to the Branyon c, Crockett sl, Falba sl, and Wilson sil soils. These data represent all values for recovery of applied P from soils incubated from 0 to 552 h. Recovery of C3P or C4P (and their hydrolysis products) applied to Falba sl was excellent at 98% for both P sources and P levels, a recovery level to be expected for a sandy loam soil. Crockett sl yielded 92% of the applied P during extraction. Hossner and Melton (33) reported recoveries of 87–95% for diP in Crockett sl using the 0.5 M H_2SO_4 extraction procedure of Gilliam and Sample (12). Unrecovered P was apparently in the form of hydrolysis products of C3P and C4P, which is more strongly retained by the soil. Subsequent extraction of selected samples with 0.5 M H_2SO_4 and 1.0 M NaOH indicated traces of diP and triP but no C3P or C4P.

Recoveries of the applied P from Wilson sil were 93.4 and 89.7% (Table 3), respectively, for treatments receiving C3P and C4P. Recovery was considered to be acceptable despite the increased difficulty in extraction compared to the sandy loam soils. Branyon c clearly presented a problem in P extraction compared to the other soils. Recovery of applied P at the lower application rate was ≤81%. Recovery of applied P to Branyon

Table 4. Retention Times for P Compounds Analyzed under Different Elution Conditions Using Ion Chromatography

phosphate species	eluent NaOH (M)				
	0.080 ^a (min)	0.085 (min)	0.090 (min)	0.080 → 0.100 ^b (min)	0.024 → 0.100 ^c (min)
monoP	2.4	2.2	2.0	2.4	6.7
diP	5.0	2.7	2.6	3.8	8.3
triP		5.1	3.6	5.4	9.2
C3P		6.3	4.1	5.8	9.6
tetraP		8.5	4.7	8.0	10.6
C4P		12.2	7.2	8.7	11.2

^a Used for monoP and diP; does not elute other P compounds well but separates monoP from sulfate. ^b NaOH gradient run from 0.080 M (0.1 min) to 0.100 M (2.0 min). ^c NaOH gradient run from 0.024 M (0.1 min) to 0.100 M (6.0 min).

c tended to decline with incubation time (data not shown). Hons et al. (34), using the method of Gilliam and Sample (12), reported total P recoveries of 92.0% for diP and 92.8% for triP in a calcareous Houston Black c, a soil very similar to Branyon c. Reextraction of Branyon c indicated traces of C3P and C4P, but the amount was consistently ≤ 0.002 g of cyclic P kg^{-1} .

Soils were selected for this study to represent a range of soil properties. Soil pH, biological activity, texture, organic C, reactive oxides of Fe and Al, and cation-exchange capacity have been reported to influence polyphosphate hydrolysis and/or P retention by soils. The extraction procedure used in this study successfully removed all cyclophosphate from the soil in the water, allowing the cyclophosphates to be protected from hydrolysis in strong acid and base solutions. Hydrolysis products associated with CaCO_3 and iron and aluminum oxides were removed from the soil in the subsequent H_2SO_4 and NaOH extractions. The cyclophosphates were combined with P removed in the H_2SO_4 and NaOH extracts once these solutions had been neutralized. The most important soil characteristics related to recovery of applied P appear to be the amount of clay and the concentrations of aluminum and iron oxides in the sample. Recovery of applied cyclophosphate generally decreased with increasing clay and aluminum and iron oxide contents, probably due to stronger sorption of hydrolysis products to the soil constituents and less efficient leaching of the clayey samples. Similar total P recovery values were obtained for sterilized and nonsterilized samples, so it was concluded that biological factors do not alter the recovery of applied cyclophosphate from these soils.

Determination of Mono- and PolyP in Soil Extracts by Ion Chromatography. Isocratic elution of P at 0.080 M NaOH, the same eluent strength used by Chino and Sato (35), was insufficient to adequately separate all P species in a reasonable amount of time. It was determined that 0.080 M NaOH was satisfactory for separating monoP from NO_3^- and SO_4^{2-} , but the elution of triP and cyclic P was poorly resolved. Further work using stronger eluents indicated that 0.085 M NaOH eluted all P species within 15 min (Table 4) but the C4P peak at 12.2 min was still poorly resolved (broad, rounded peak). A further increase in NaOH concentration to 0.090 M eluted chain and cyclic P in good time, although some monoP was eluted with SO_4^{2-} . Peaks were well-defined (sharper) due to the higher eluent concentration. Gradient elution from 0.024 to 0.100 M NaOH resulted in all peaks being sharp and resolved, with monoP being eluted after the SO_4^{2-} peak (Table 4). Sekiguchi et al. (36) used an eluent gradient of 0.03–0.20 M NaOH to separate polyphosphates from P1 to P52 over 30 min at a flow rate of 0.5 mL min^{-1} .

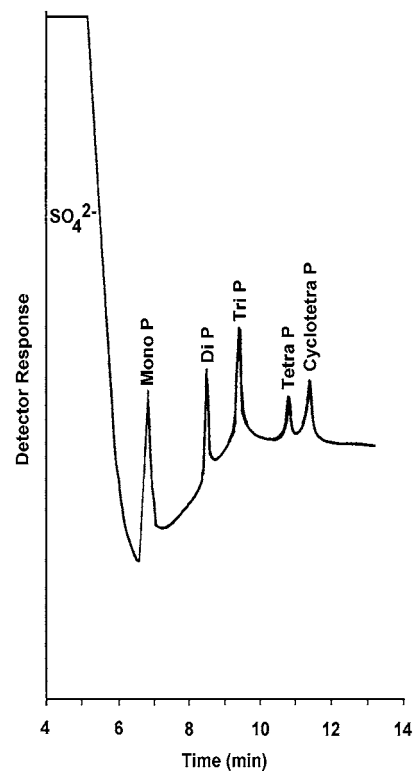


Figure 4. Gradient ion chromatogram of speciated soil P extracted from Falba sl soil (0.400 g of P g^{-1} of soil) after incubation at 0.033 MPa moisture content and 20 °C for 14 days. NaOH gradient was pumped at 1.5 mL min^{-1} from 0.024 M (0.1 min) to 0.100 M (6 min).

A representative gradient chromatogram of an extract from Falba soil (0.400 g of C4P kg^{-1} of soil) after a 20 °C incubation for 14 days is presented in Figure 4. The extract contains SO_4^{2-} and P in the forms of monoP, diP, triP, tetraP, and C4P. Recovery of the P applied as C4P was 99.2%. The individual P species were clearly separated and quantified. Detection limits for analysis of P compounds by gradient elution chromatography was from 0.05 to 0.10 mg of P L^{-1} for pure solutions and from 0.10 to 0.15 mg of P L^{-1} for soil extracts. Detection limits for monoP using IC have been reported at 0.076 mg of P L^{-1} in soil extracts when large injection volumes (2 mL) were used (37). Dick and Tabatabai (38) reported a detection limit of 0.02 $\text{mg of monoP L}^{-1}$ for IC analysis of natural waters.

Dilute hydroxide solutions have been used increasingly as eluents in gradient ion chromatographic separation with suppressed conductivity detection because they produce a low detection background (39, 40). Recently, Sekiguchi et al. (36) reported the simultaneous determination of condensed phosphates in food products with sodium hydroxide (NaOH) eluent containing 15% methanol. This method can be used to determine linear polyphosphates and cyclic phosphates; however, the method has some practical problems. Sekiguchi et al. (36) have suggested the use of ion chromatography in conjunction with the EG40 hydroxide eluent generator for better precision in the analysis of condensed phosphates in food samples.

Conclusions. Cyclotetraphosphate was prepared with good yield and desirable physical properties that permit its use in future soil and plant research. Cyclotetra- and cyclotriphosphate and their hydrolysis products were prepared to >99% purity. A sequential extraction procedure (water/ H_2SO_4 /NaOH) for removal of cyclic phosphates and their reaction products from the soil with minimal hydrolysis proved to be satisfactory for sandy loam and silty loam soils. Recovery of applied P from a

calcareous clay soil was less satisfactory. Gradient ion chromatography using conductivity detection proved to be a rapid and sensitive technique for the separation and direct analysis of cycloP and their hydrolysis products in soil extracts. Samples from cycloP preparation and purification or soil extraction could be readily analyzed in 8–13 min. The method has some limitations due to the high amount of anion in soil extracts and the steep eluent gradient needed to separate monoP from the SO_4^{2-} contained in the soil extract.

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