**MONTANA STATE UNIVERSITY**

Precipitation of calcium phosphates in the presence of soluble organic matter
by Paul Rudolf Grossl

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University
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Abstract:
Dicalcium phosphate dihydrate (DCPD) and octacalcium phosphate (OCP) are important P solid phases in soil systems. They both have been recognized as precursor phases to the formation of thermodynamically more stable hydroxyapatite (HAP). Their metastability with respect to HAP may be explained by precipitation kinetics and the influence of dissolved organic C (DOC) on crystal growth. Precipitation rates of DCPD and OCP were measured at respective pHs of 5.7 and 6.0, and 25°C in the absence and presence of organic acids common to soil solutions using a seeded crystal growth method. Initial total soluble (TS) C levels added as humic, fulvic, tannic, and citric acids ranged from 0.26 to 9.0 mM C for DCPD experiments and 20 uM to 2 mM C for OCP experiments. Precipitation of DCPD and OCP was inhibited by adsorption of these organic acids onto crystal seed surfaces blocking sites that act as nuclei for new crystal growth. The efficiency of adsorbed organic acids at inhibiting DCPD and OCP crystal growth is related to their functional group content, size, hydrophobicity, geometry, and orientation on the crystal surface. Of the three Ca phosphate minerals considered, precipitation inhibition increased in the order DCPD < OCP < HAP.

Soil incubation and plant available P experiments were conducted to examine the influence of organic acids on P status of soil systems. For the incubation study, a soil was treated with solutions containing 6 to 8 mM CaTS and PO4TS, with and without 2 mM C as humic acid. Studies examining bioavailability of P to spring wheat (Triticum aestivum L.) and sorghum-sudan grass (Sorghum Sudanese L.) were carried out in controlled environment chambers on soils treated with organic acids. These experiments demonstrated that organic acids were able to increase P solubility and plant availability in soils.

Fulvic acid isolated from a water soluble wheat straw extract was chemically characterized and was most similar to fulvic acids isolated from aquatic systems. It was representative of the hydrophobic, nonhumified fraction of the initial leachate of plant residues and found to inhibit Ca phosphate precipitation. Thus, plant residue breakdown products can enhance P fertilizer efficiency by increasing P bioavailability in soils.

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A thesis submitted in partial fulfillment
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# APPROVAL

of a thesis submitted by

Paul Rudolf Grossl

This thesis has been read by each member of the graduate committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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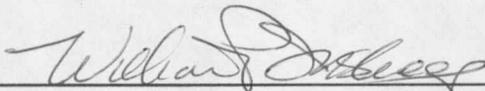
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# ABSTRACT

Dicalcium phosphate dihydrate (DCPD) and octacalcium phosphate (OCP) are important P solid phases in soil systems. They both have been recognized as precursor phases to the formation of thermodynamically more stable hydroxyapatite (HAP). Their metastability with respect to HAP may be explained by precipitation kinetics and the influence of dissolved organic C (DOC) on crystal growth. Precipitation rates of DCPD and OCP were measured at respective pHs of 5.7 and 6.0, and 25°C in the absence and presence of organic acids common to soil solutions using a seeded crystal growth method. Initial total soluble (TS) C levels added as humic, fulvic, tannic, and citric acids ranged from 0.26 to 9.0 m$M$ C for DCPD experiments and 20 u$M$ to 2 m$M$ C for OCP experiments. Precipitation of DCPD and OCP was inhibited by adsorption of these organic acids onto crystal seed surfaces blocking sites that act as nuclei for new crystal growth. The efficiency of adsorbed organic acids at inhibiting DCPD and OCP crystal growth is related to their functional group content, size, hydrophobicity, geometry, and orientation on the crystal surface. Of the three Ca phosphate minerals considered, precipitation inhibition increased in the order DCPD < OCP < HAP.

Soil incubation and plant available P experiments were conducted to examine the influence of organic acids on P status of soil systems. For the incubation study, a soil was treated with solutions containing 6 to 8 m$M$ $Ca_{TS}$ and $PO_{4TS}$, with and without 2 m$M$ C as humic acid. Studies examining bioavailability of P to spring wheat (*Triticum aestivum* L.) and sorghum-sudan grass (*Sorghum sudanese* L.) were carried out in controlled environment chambers on soils treated with organic acids. These experiments demonstrated that organic acids were able to increase P solubility and plant availability in soils.

Fulvic acid isolated from a water soluble wheat straw extract was chemically characterized and was most similar to fulvic acids isolated from aquatic systems. It was representative of the hydrophobic, nonhumified fraction of the initial leachate of plant residues and found to inhibit Ca phosphate precipitation. Thus, plant residue breakdown products can enhance P fertilizer efficiency by increasing P bioavailability in soils.

# CHAPTER 1

## INTRODUCTION

When phosphorus fertilizers are applied to soils, chemical processes occur that "fix" or retain P, decreasing phosphate activity. Mechanisms responsible for P fixation are precipitation of P as Ca, Fe, and Al phosphates, adsorption of $PO_4$ onto oxides, clays, carbonates, and soil organic matter, and immobilization into biomass P. These processes limit the effectiveness of P fertilizer applications; therefore, further applications are necessary to sustain desired crop productivity.

At pHs ranging from near neutral and above, Ca phosphates including dicalcium phosphate dihydrate (DCPD), octacalcium phosphate (OCP), and hydroxyapatite (HAP) are important P solid phases in soils (Lindsay, 1979). Dicalcium phosphate dihydrate and OCP are important reaction products of P fertilizers in soil and are precursors to the formation of thermodynamically more stable HAP. Usually, HAP is predicted to control $PO_4$ activity in soil solutions (Lindsay, 1979; Stumm and Morgan, 1981); however, DCPD and OCP have been found to persist and control $PO_4$ activity for considerable lengths of time (Bell and Black, 1970; Fixen et al., 1983; Moreno et al., 1960). The metastability of DCPD and OCP with respect to HAP, whose formation is relatively slow, indicates that precipitation kinetics play a major role in controlling $PO_4$ levels in soil solutions.

The presence of organic matter may also influence the phosphate chemistry in soil environments. Soluble organic matter was found to be responsible for the persistence of more soluble DCPD and a reduction in the rate of formation of less soluble octacalcium phosphate (OCP) in soils (Moreno et al., 1960). Similarly, numerous studies have demonstrated that the addition of organic amendments, such as sewage sludge and manure, to calcareous soils resulted in higher aqueous $PO_4$ concentrations than lone additions of inorganic phosphate (O'Connor et al., 1986; Meek et al., 1979; Abbott and Tucker, 1973).

Conservation tillage practices are associated with an accumulation of plant residue and organic C at the soil surface (Dick, 1983; Doran, 1980; Weil et al., 1988). The organic C of plant residues is comprised of: (i) materials present in the initial leachate of plant litter consisting mostly of carbohydrates that degrade rapidly and colored organic acids with molecular weight < 1000 (Thurman, 1985), and (ii) residue humification products consisting of humic and fulvic acids (Shields and Paul, 1973; Stott et al., 1983; Thurman, 1985). These organic constituents appear to play an important role in increasing the efficiency of P fertilizers. Weil et al. (1988) suggested that the increased organic matter under no-till practices may enhance P availability by blocking P adsorption sites on soil colloids. Organic anions commonly found in the rhizosphere compete with phosphate for adsorption sites on oxide and clay surfaces (Nagarajah et al., 1970; Lopez-Hernandez et al., 1986). For example, certain organic molecules (malate and oxalate) are more rapidly adsorbed to soil surfaces than

phosphate, which can improve the phosphate status of the soil (Lopez-Hernandez et al., 1986).

Organic acids commonly found in soil solutions have also been shown to inhibit the precipitation of Ca phosphates (Inskeep and Silvertooth, 1988a). The probable mechanism of inhibition is adsorption of organic acids onto crystal surfaces, which block sites acting as nuclei for new crystal growth. In natural environments, potential inhibitors of crystal growth such as soluble organic matter are ubiquitous. Consequently, precipitation inhibition may be an important mechanism in explaining the metastability of more soluble Ca phosphate solid phases (DCPD and OCP) with respect to more thermodynamically stable HAP.

The objectives of this investigation were to: (i) examine the effects of naturally occurring organic acids on DCPD and OCP precipitation; (ii) determine mechanisms of inhibition by which these organic ligands (humic, fulvic, tannic, and citric acids) influence the rates of DCPD and OCP precipitation; (iii) examine the role of precipitation inhibition of Ca phosphates in soils and its effect on P availability to plants; and (iv) chemically characterize fulvic acid isolated from a water soluble extract of wheat straw.

# CHAPTER 2

## PRECIPITATION OF DICALCIUM PHOSPHATE DIHYDRATE

## IN THE PRESENCE OF ORGANIC ACIDS

### Introduction

Dicalcium phosphate dihydrate (DCPD) is an important reaction product of P fertilizer applications to calcareous soils and has been recognized as a precursor in the formation of thermodynamically more stable hydroxyapatite (HAP). Although HAP is generally predicted to control $PO_4$ activity in soil solutions (Lindsay, 1979; Stumm and Morgan, 1981), equilibrium $PO_4$ levels are frequently regulated by DCPD and octacalcium phosphate (OCP), both metastable to HAP (Moreno et al., 1960; Fixen et al., 1983; Bell and Black, 1970). Since DCPD and OCP can persist and control $PO_4$ activity in soils for considerable lengths of time (Moreno et al., 1960; Bell and Black, 1970) and formation of HAP is relatively slow, precipitation kinetics may be paramount in determining $PO_4$ levels in soils.

Phosphate chemistry in soil environments may also be influenced by the presence of organic matter. Additions of organic amendments (sludge and manure) to calcareous soils reportedly increase $PO_4$ solubility over time more than lone additions of inorganic phosphate (O'Connor et al., 1986; Meek et al., 1979; Abbott and Tucker,

1973). Similarly, Moreno et al. (1960) found that soluble organic matter was responsible for the persistence of DCPD and the reduction in the rate of formation of OCP in calcareous soils. Inskeep and Silvertooth (1988a) discovered that HAP precipitation was inhibited by organic acids common to soil environments. These organic acids inhibited HAP precipitation rates by adsorbing onto crystal seeds that act as nuclei for new crystal growth. Organic constituents may inhibit formation of thermodynamically stable calcium phosphates, thereby maintaining higher $PO_4$ levels in soils.

To better understand control of $PO_4$ activity in soils, and since there have been no investigations on the direct effect of soil organic acids on DCPD precipitation rates, the objectives of this study were to: (i) examine the effect of several soil organic acids (humic, fulvic, tannic, and citric) on DCPD precipitation; and (ii) determine the mechanism of inhibition by these organic acids on DCPD precipitation rates.

## Materials and Methods

### Organic Acids

The water soluble organic acids used in the DCPD precipitation experiments consisted of a peat humic acid obtained from the International Humic Substances Society (IHSS), a fulvic acid isolated from a water soluble extract of wheat straw (WSE-FA) using methods described by Thurman and Malcolm (1981) and Leenheer (1981), and commercially purchased tannic and citric acids (J.T. Baker Chemical Company, Phillipsburg, NJ). Elemental analysis of the WSE-FA was performed by

Huffman Laboratories (Golden, CO; Table 1). Total acidity and carboxylic acid functional group content were determined using barium hydroxide and calcium acetate methods, respectively (Schnitzer, 1982). Stock solutions of the above organic acids containing 0.01 $M$ $C_{TS}$ (TS=total soluble) were prepared in distilled-deionized water not more than two days prior to conducting experiments and were stored at 5°C.

Table 1. Elemental composition and functional group content of IHSS peat humic acid and wheat straw extract fulvic acid.

| Element/functional group | Fulvic | Humic |
|---|---|---|
| | ――――――― g kg$^{-1}$ ――――――― | |
| C | 550.0 | 565.0 |
| O | 357.0 | 354.0 |
| H | 52.0 | 41.0 |
| N | 8.5 | 37.1 |
| S | 4.6 | 7.0 |
| P | <1.0 | 0.2 |
| Ash | 24.4 | 9.0 |
| | ――――――― mmol c g$^{-1}$ ――――――― | |
| COOH | 5.5 | 4.2 |
| ∅−OH | 0.7 | 4.6 |

Crystal Growth Experiments

The DCPD precipitation experiments were conducted in a flat-bottomed, water-jacketed 600 cm$^3$ vessel covered with a removable plexiglass lid containing entry ports for a stirrer, burette tip, pH and Ca ion electrodes, and N$_2$ gas bubbler.

The DCPD precipitation rates were measured at constant pH = 5.7 and temperature = 25°C using a seeded crystal growth method (Marshall and Nancollas, 1969). Starting supersaturated solutions consisted of 400 mL of 13.0 m$M$ Ca$_{TS}$, 11.3 m$M$

$PO_{4TS}$. These solutions also contained 20 m$M$ KCl as a background ionic strength commonly found in soil solutions. The level of initial supersaturation with respect to DCPD was 3.5 (ion activity product/solubility product ($K_{sp}$) where log $K_{sp}$ = −6.57 (Bennett and Adams, 1976)). Solutions were stirred with a three-bladed polyethylene propeller driven by an overhead motor at 6.17 rev s$^{-1}$. Humidified $N_2$ gas was bubbled through solutions at 3.3 cm$^3$s$^{-1}$ to purge $CO_2$(g). Solutions were brought to pH 5.7 by dropwise addition of 0.1 $M$ HCl and 0.1 $M$ KOH and were allowed to stabilize for 30 minutes prior to the introduction of 300 mg DCPD seed crystals. The DCPD seed crystals were prepared by aging Baker reagent grade DCPD under $N_2$(g) for four weeks. Seed crystals had a surface area (S) = 3.0 m$^2$g$^{-1}$ as measured by a triple point $N_2$-Brunauer-Emmett-Teller adsorption isotherm. Total dissolution of three DCPD seed crystal subsamples resulted in a Ca/P ratio = 0.980 ± 0.005 (theoretical Ca/P for DCPD is 1.0). DCPD seed crystals were also characterized by X-ray diffraction and scanning electron microscopy (SEM). X-ray diffraction patterns displayed only those peaks characteristic of DCPD (Smith, 1967).

A pH-stat titrator maintained constant solution pH by addition of 0.4993 $M$ KOH which was continuously recorded throughout all experiments. Five mL samples were periodically collected during each precipitation experiment, filtered through prewashed 0.2 micron filters (Gelman GA-8, Gelman Sciences, Inc., Ann Arbor, MI), and analyzed for $Ca_{TS}$ by atomic absorption spectroscopy and $PO_{4TS}$ using a modified Murphy-Riley colorimetric method (Harwood et al., 1969). Seed crystals were collected after several experiments and analyzed by X-ray diffraction and SEM to

confirm the formation of DCPD. Average stoichiometries for the change in $Ca_{TS}$, $PO_{4TS}$, and $[OH^-]$ during precipitation were also close to values predicted from the overall stoichiometry for DCPD precipitation at pH 5.7 (Eq. [1]; Table 2).

Table 2. Experimental and predicted stoichiometric ratios based on DCPD precipitation at pH 5.7.

| Element ratio | Experimental | Theoretical |
|---|---|---|
| Ca/P | $1.01 \pm 0.07$† | 1.00 |
| Ca/OH | $1.07 \pm 0.03$ | 1.06 |
| P/OH | $1.07 \pm 0.06$ | 1.06 |

† $\pm$ values are standard deviations (three experiments).

Experiments run in the presence of organic acids had $C_{TS}$ levels ranging from 1.1 to 7.8 m$M$ as fulvic acid, 0.26 to 2.05 m$M$ as humic acid, 0.9 to 9.0 m$M$ as tannic acid, and 0.5 to 8.0 m$M$ as citric acid. Final $C_{TS}$ values were measured using a C-analyzer (Dohrmann CD-80, Santa Clara, CA). Calcium ion activity was measured before and after each experiment with a $Ca^{2+}$ ion electrode (Radiometer #f2112, Copenhagen, Denmark) to determine the degree of complexation of $Ca^{2+}$ with organic acids. Experiments were also run at 13, 19, 25, and 32°C to calculate an Arrhenius activation energy ($E_a$) for DCPD precipitation.

Determination of Rate Constants

The overall stoichiometry predicted for the DCPD precipitation reaction at pH 5.7 and 25°C is:

$$Ca^{2+} + 0.94\ H_2PO_4^- + 0.06\ HPO_4^{2-} + 2H_2O = CaHPO_4 \cdot 2H_2O + 0.94H^+ \quad [1]$$
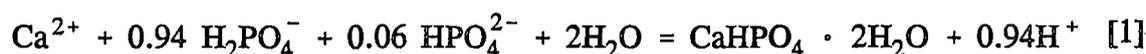
The forward rate constant ($k_f$) was determined using the second order rate law expression for the rate of formation of DCPD (Marshall and Nancollas, 1969),

$$\text{Rate} = \frac{-d[Ca^{2+}]}{dt} = \frac{d[DCPD]}{dt} = k_f S [Ca^{2+}][HPO_4^{2-}]\gamma_2^2 \qquad [2]$$

where brackets represent concentrations, $\gamma_2$ is the divalent ion activity coefficient determined from the Davies equation, and S is the surface area of DCPD seed crystals (2.28 $m^2L^{-1}$). The rate of precipitation was determined by monitoring $[Ca^{2+}]$ over time ($-d[Ca^{2+}]/dt$) which is equal to the DCPD precipitation rate, $d[DCPD]/dt$.

The stoichiometry of the precipitation reaction (Eq. [1]) can be used to relate the loss in soluble Ca to the loss in soluble $PO_4$ over time:

$$Ca_{TS_0} - Ca_{TS_x} = PO_{4TS_0} - PO_{4TS_x} \qquad [3]$$

where the subscripts 0 and x denote concentrations at time = 0, and concentrations at time = x. Eq. [3] can be solved for the concentration of $HPO_4^{2-}$ utilizing distribution coefficients ($\alpha$) for the $Ca^{2+}$ and $H_2PO_4^-$ species:

$$[HPO_4^{2-}]_x = \alpha_{H_2PO_{4x}^-}\left[\frac{[HPO_4^{2-}]_0}{\alpha_{H_2PO_{40}^-}} - e\left(\frac{[Ca^{2+}]_0}{\alpha_{Ca_0^{2+}}} - \frac{[Ca^{2+}]_x}{\alpha_{Ca_x^{2+}}}\right)\right] \qquad [4]$$

($e = \gamma_1 \cdot \gamma_2^{-1} \cdot 10^{-7.2} \cdot 10^{pH}$). Substituting Eq. [4] into Eq. [2] results in the expression:

$$\frac{d[Ca^{2+}]_x}{a[Ca^{2+}]_x^2 + b[Ca^{2+}]_x} = k_f Sdt \qquad [5a]$$

where $a = \dfrac{\alpha_{H_2PO_{4x}^-}}{\alpha_{Ca_x^{2+}}} \cdot e \cdot \gamma_2^2,$ \qquad [5b]

$$b = \alpha_{H_2PO_{4_x}^-} \cdot \gamma_2^2 \left[ \frac{[HPO_4^{2-}]_0}{\alpha_{H_2PO_{4_0}^-}} - e \cdot \frac{[Ca^{2+}]_0}{\alpha_{Ca_0^{2+}}} \right] \qquad [5c]$$

which upon integration yields

$$\frac{1}{b} \ell n \frac{a[Ca^{2+}]_x}{a[Ca^{2+}]_x + b} = k_f St + C \qquad [6]$$

where C = constant of integration.

The gamma and alpha terms used to derive "a" (Eq. [5a]) and "b" (Eq. [5b]) in Eq. [6] were calculated over the course of the reaction using the chemical equilibrium model GEOCHEM (Sposito and Mattigod, 1979; Parker et al., 1987) from analytically determined $Ca_{TS}$, $PO_{4TS}$, $K_{TS}$, and $Cl_{TS}$ input values and thermodynamic constants listed in Inskeep and Silvertooth (1988b). In a typical precipitation experiment at 25°C in the absence of organic acids, ionic strength declined from 0.07 to 0.06 (approximately 14%). This results in small changes in $\gamma_1$ from 0.80 to 0.81 and $\gamma_2$ from 0.41 to 0.43 (1 and 4% changes, respectively). Changes in $\gamma_1$, $\gamma_2$, and the distribution coefficients used in Eq. [5b] and [5c] as a function of time resulted in slight changes in a and b (< 10%). Although these changes are relatively minor, a and b were calculated over the course of the reaction to reflect changes in solution composition.

Calcium concentrations were calculated using the relationship

$$[Ca^{2+}]_x = [Ca^{2+}]_0 - 1.06 \left([OH^-]_x - [OH^-]_0\right) \qquad [7]$$

based on the overall stoichiometry for DCPD precipitation at pH 5.7 (Eq. [1]), where $[Ca]_o$ = initial free $Ca^{2+}$ concentration at time = 0, calculated by GEOCHEM. This method allowed for the determination of an essentially endless number of $Ca^{2+}$ data points (we used 15 to 25) obtained from continuous plots of $OH^-$ vs. time, compared to only 4 to 8 $Ca^{2+}$ values actually measured from subsamples withdrawn from the reaction vessel. Values calculated using Eq. [7] differed by an average of only 7% from those determined by GEOCHEM (values obtained from three experiments). Further justification for using Eq. [7] was that Ca/OH ratios determined experimentally were consistent with the ratio predicted from the overall stoichiometry of DCPD precipitation (Eq. [1]; Table 2).

Forward rate constants were determined from the slopes of integral vs. time plots (Eq. [6]). For example, the initial 14 to 16 points representing the first 12 to 20 minutes of DCPD precipitation were used to calculate $k_f$ for experiments run in the absence of organic acids at 25°C. During this time, the majority of DCPD precipitation had occurred, as indicated by the loss of $Ca_{TS}$ and $PO_{4TS}$ and the addition of $[OH^-]$ (Figure 1).

Carbon Adsorption

Adsorption data for tannic and citric acids were determined by measuring $C_{TS}$ on samples collected at the end of precipitation experiments using a C-analyzer. A separate C adsorption study was established using the humic and fulvic acids to confirm their adsorption onto DCPD crystal seed surfaces. These experiments were