

HYDROLYSIS OF 4-NITROPHENYL PHOSPHATE BY MAGNETITE OR FLY ASH: A MODEL FOR THE FATE OF ORGANOPHOSPHATE CONTAMINANTS IN THE SOIL ENVIRONMENT

Fikru Tafesse*, B.S. Nakani and Ntsapokazi C. Deppa

Chemistry Department, University of Transkei, Private Bag X1, UNITRA, Umtata 5117,
Republic of South Africa

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ABSTRACT. Reaction of fly ash or magnetite towards hydrolysis of 4-nitrophenyl phosphate (NPP) was investigated in both the solid and solution phases at 10^{-3} M concentration in oil-in-water microemulsions and aqueous media. The reactions were monitored by measuring the absorbance of the nitrophenolate ion produced in the reaction aliquots with time. The possible application of the above systems for environmental decontamination of organophosphates is discussed. The study provides the basis for increasing our knowledge of the fate of persistent organophosphates in the soil and provides a possible application to environmental clean-up problems.

KEY WORDS: Environmental clean-up, Microemulsion media, Magnetite, Fly ash, Hydrolysis of organophosphates, Nitrophenyl phosphate

INTRODUCTION

A contaminant is defined as a substance that is not natural to the soil, or a naturally occurring substance, which has undesirable effects if its concentration exceeds a certain threshold. The potential for human activities to contaminate the environment has become of increasing concern during the past two decades as a result of increased fertilizer and pesticide use. There is a need for more information on the occurrence and fate of organophosphorous insecticides in surface water, soil, and ground water and their impact on plants, invertebrates and mammals. In addition, organophosphates and carbamates usually contain solvents such as petroleum distillates which may be listed as inert ingredients but which can produce toxic effects in any accidental ingestion.

Much consideration is given to reduce pollution caused by organophosphate esters that are resistant to biological degradation and can accumulate in the biosphere and biological organisms. In the past, most approaches to handle pollution could be summed up by the phrase "dilution is the solution to pollution". However, pollution levels have increased so much in amount and toxicity that this approach is no longer acceptable. The most versatile decontamination method is washing or spraying with water that contains soap or detergent. A serious drawback to this method is that although most contaminants are removed and diluted, not all of them are neutralized or destroyed. To neutralize and destroy the organophosphate contaminants bleach or other chemical agents must be added [1]. The investigation with real organophosphate insecticides was not undertaken for safety reasons. However, nitrophenyl phosphate was utilized as a model system. Degradation of parathion produces paraoxon which is more toxic and persistent than the parent compound. Our model system closely resembles paraoxon. Hence conclusions drawn for hydrolysis of 4-nitrophenylphosphate will be applicable to those of paraoxon which is the dominant intermediate oxidative product of parathion. Earlier studies [2] showed that interaction of organophosphate pesticides with soils depend on the soil type. Metal ion catalysis for the hydrolysis of organophosphates has been the subject of previous

*Corresponding author. E-mail: Tafesse@getafix.utr.ac.za

reports [3-5]. Much effort has been directed on the mechanism of catalysis and the effect of the decontamination media [6]. The physical and chemical properties of organophosphates must be taken into account for of an effective decontaminant media. Effective decontamination requires the rapid dissolution of agents in the media. For this reason decontamination media based on non-aqueous, mixed solvent, emulsion, microemulsion and self-organizing surfactant assembly seem to be worth investigating. Though most agents are soluble in non-aqueous media, the formation of large amounts of organic waste is unacceptable. Mixed solvent systems suffer from slow oxidation and substitution reaction as the polarity of the solvent decreases. The microemulsion and micellar systems showed better promise as reported in previous investigation [6]. In the present study, a system containing metal ions in microemulsion media has been chosen as the decontaminant in both the solid and solution phases. Ferrous and ferric ions were utilized and a comparative study was conducted in both aqueous and microemulsion media. The microemulsion-based system is simple, inexpensive, mild and relatively rapid. These features arise from the fact that microemulsion represent a community of molecules that function only by virtue of cooperative action. Each of the components (water, hydrocarbon, surfactant, cosurfactant, organophosphate agent and the metal ion) has its own residence site and its own role in the reaction. This kind of self-assembly, inherent to microemulsions, results in the formation of an organized system that helps in assisting hydrolysis of organophosphates.

The use of fly ash and magnetite with 4-nitrophenylphosphate as model systems will provide the basis for increasing our knowledge of the fate of persistent organophosphates in the soil. As far as the mineral component of the soil is concerned, the minerals commonly of great abundance are layers of silicates. The investigation will shed some light on the possible use of the above materials as decontaminants of organophosphates in the soil.

EXPERIMENTAL

All reagents used were either analytical-reagent grade or the purest grade available commercially and were used without further purification. Measurement of pH was made with a Crison (Barcelona) pH meter Model No. 2002, using a combination electrode. A Philips UV/VIS spectrometer Model No. Pu 8620 was used to obtain spectra and collect rate data. The pH of the reaction mixture was maintained by adding drops of NaOH or HClO₄ from a glass rod (glass stick dotting). 4 beakers containing 4 M, 2 M, 0.5 M and 0.1 M NaOH or HClO₄ with different sizes of glass rods were assembled and used to stabilize the required reaction pH. Microemulsions were prepared [7] by mixing hexane, sodium dodecylsulfate (SDS) and butan-1-ol and titrating the slurry with water, agitating mildly to give a clear solution. The following compositions (by mass) were used: ME1, H₂O (90%)–hexane (2%)–SDS (2%)–butan-1-ol (6%); ME2, H₂O (82%)–hexane (3%)–SDS (5%)–butan-1-ol (10%); ME3, H₂O (60%)–hexane (4%)–SDS (18%)–butan-1-ol (18%); ME4, H₂O (43.2%)–hexane (10.4%)–SDS (14.8%)–butan-1-ol (31.6%). The ME2 formulation was utilized in this study since previous investigation showed maximum hydrolysis of organophosphates in ME2 reaction solutions [8]. Ferrous ammonium sulfate and ferric ammonium sulfate were used for Fe(II) and Fe(III), respectively. Turnbull's blue was synthesized from potassium hexacyanoferrate(II) using known procedures [9, 10]. Magnetite was prepared by roasting steel wool at 1200 °C for about 5 hours. Dissolution of magnetite was achieved in small amounts of concentrated hydrochloric acid. Fly ash was obtained from South African Coal Ash Association (SACAA) and was used without further purification. Partial dissolution of fly ash was achieved in small amounts of concentrated hydrochloric acid. The protocol for the study consists of mixing 8 mL of a 5 × 10⁻³ M solution of 4-nitrophenyl phosphate (NPP) in a thermostated reaction vessel with 4 mL of NaClO₄ (1 M) and 20 mL of the solvent. The solvent was either water or one of the microemulsions. The pH of the solution was adjusted to 6.5. An 8-mL volume of temperature equilibrated metal ion solution

(5×10^{-3} M), pH 6.5, hydroxoqua form predominant $[\text{ML}_4(\text{OH})(\text{H}_2\text{O})]$ where L is a ligand or water acting as a ligand in the case of free metal ion solutions, was then added, making the total volume to 40 mL. Aliquots (2 mL) were withdrawn from the reaction vessel at 0, 1, 3, 5, 10, 15, 30, and 60 min, respectively, and mixed with 2 mL of phosphate buffer (pH 7.4). The buffer system was prepared by mixing 8.62 g of sodium phosphate dibasic and 5.42 g of sodium phosphate monobasic salts and making up to 1000 mL with carbon dioxide free, distilled water. The absorbance of the reaction solution was then measured at 400 nm after mixing. The amount of nitrophenolate produced was determined from the calibration curve and the percentage hydrolysis calculated, assuming 100% nitrophenolate production for complete hydrolysis. In an unbuffered solution the acid–base reaction of *p*-nitrophenol changes in concentration results in a shift in the equilibrium position and a consequent deviation from Beer's law. In buffer solution, the pH would be fixed and the ratio of the concentration of *p*-nitrophenolate to the *p*-nitrophenol would be constant thereby obeying Beer's law. The advantages of this technique over direct spectrophotometric determination of phosphates on the basis of the reaction of heteropoly yellow, heteropoly blue and their ion associates with cationic dyes is that its analysis time is short and the sensitivity is high. Above all the method does not require addition of extra quenching reagents to release the bound species as has been reported previously [11-13]. All absorbances were taken against water blank. Interference from other compounds in the analyte was not detected. The molar absorption coefficient was determined from calibration curve studies with standard *p*-nitrophenol solutions as $1.118 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ and the system confirmed to Beer's law over the concentration range 0–20 $\mu\text{g L}^{-1}$ of nitrophenol when measured at 400 nm in a 1 cm cell.

RESULTS AND DISCUSSION

The results of this investigation are given in Table 1. The values for the different systems are average values of quadruplet studies at 25 °C. The standard deviation for the four assays ranged from 0.01 to 0.04 in each case.

The degradation of the model compound 4-nitrophenylphosphate in the absence of metal ions using the same protocol was negligible. A maximum of 0.5% production of nitrophenol was noted after 30 minutes of reaction time.

A summary of the results of our investigation is depicted in Figure 1. Only the values after three minutes of reaction time are considered in the graph. Scheme 1 shows the alternative degradation pathway for the hydrolysis of parathion with metal ions.

Table 1. Hydrolysis of 4-nitrophenyl phosphate by several metal ions at different NPP to metal ratio in aqueous and microemulsion media. (NPP = 10^{-4} M, $I = 0.1$ M KClO_4 , 25 °C, pH 6.5).

Systems	Medium	(% Nitrophenol produced with time in minutes)					
		1	3	5	10	15	30
(NPP + metal)	Water	5.15	5.58	5.77	5.96	6.04	6.17
NPP + Fe ²⁺	Water	10.47	10.77	11.09	11.15	11.26	11.26
NPP + 2Fe ²⁺	Water	13.08	13.49	13.74	13.77	15.68	15.08
NPP + 3Fe ²⁺	Water	13.94	15.60	15.91	16.85	14.60	11.39
NPP + 4Fe ²⁺	Water	6.92	7.12	7.37	7.38	7.67	7.71
NPP + Fe ²⁺	ME2	11.15	11.33	11.46	11.60	11.83	11.87
NPP + 2Fe ²⁺	ME2	14.19	14.92	15.06	15.15	16.35	17.25
NPP + 3Fe ²⁺	ME2	15.13	15.98	16.52	17.38	16.48	16.60
NPP + 4Fe ²⁺	ME2	11.25	11.30	11.30	11.30	11.87	12.83
NPP + Fe ³⁺	Water	14.38	14.72	15.13	15.26	15.77	15.42
NPP + 2Fe ³⁺	Water	16.23	17.08	18.23	18.26	17.58	17.72
NPP + 3Fe ³⁺	Water	16.79	17.40	18.26	16.79	17.28	17.60
NPP + 4Fe ³⁺	Water	12.83	13.25	13.50	13.77	13.77	13.65
NPP + Fe ³⁺	ME2	16.25	17.10	17.81	17.71	17.15	17.15
NPP + 2Fe ³⁺	ME2	16.79	17.58	18.75	18.81	17.96	18.06
NPP + 3Fe ³⁺	ME2	17.75	18.02	18.71	18.15	18.25	18.33
NPP + 4Fe ³⁺	ME2	16.21	16.94	17.49	18.91	20.47	20.77
NPP + Fe ²⁺ /Fe ³⁺	Water	18.40	18.92	20.09	19.21	24.75	17.89
NPP + 2Fe ²⁺ /Fe ³⁺	Water	17.75	17.83	18.23	22.42	19.48	20.44
NPP + Fe ²⁺ /Fe ³⁺	ME2	18.85	19.19	22.87	26.87	19.38	19.33
NPP + 2Fe ²⁺ /Fe ³⁺	ME2	12.89	13.00	13.17	13.09	13.04	13.00
NPP + Magnetite (solution form)	Water	17.04	17.15	18.66	18.79	18.79	18.85
NPP + 2Magnetite (solution)	Water	8.85	9.10	9.21	9.79	9.25	9.40
NPP + Magnetite (solution form)	ME2	13.71	13.77	13.44	13.52	13.75	13.73
NPP + 2Magnetite (solution)	ME2	9.36	9.52	9.36	9.20	9.08	9.10
NPP + Magnetite (solid form)	Water	9.43	9.52	8.50	8.19	8.22	8.20
NPP + 2Magnetite (solid form)	Water	9.17	9.19	9.14	9.14	9.02	9.07
NPP + Magnetite (solid form)	ME2	9.19	9.52	9.29	9.27	8.52	8.31
NPP + 2Magnetite (Solid form)	ME2	4.63	5.55	5.25	3.73	5.14	5.23
NPP + Fly ash (solution form)	Water	4.79	5.95	4.93	5.14	5.23	5.09
NPP + 2Fly ash (solution form)	Water	5.13	6.20	5.90	4.92	4.70	4.75
NPP + Fly ash (solution form)	ME2	5.20	6.65	6.12	4.73	4.67	4.52
NPP + 2Fly ash (solution form)	ME2	4.19	4.84	4.68	4.39	4.32	4.10
NPP + Fly ash (solid form)	Water	4.35	4.95	4.13	4.10	4.06	4.04
NPP + 2Fly ash (solid form)	Water	4.91	4.95	4.00	4.20	4.64	4.76
NPP + Fly ash (solid form)	ME2	4.85	4.95	4.40	4.54	4.64	4.54
NPP + 2Fly ash (solid form)	ME2						

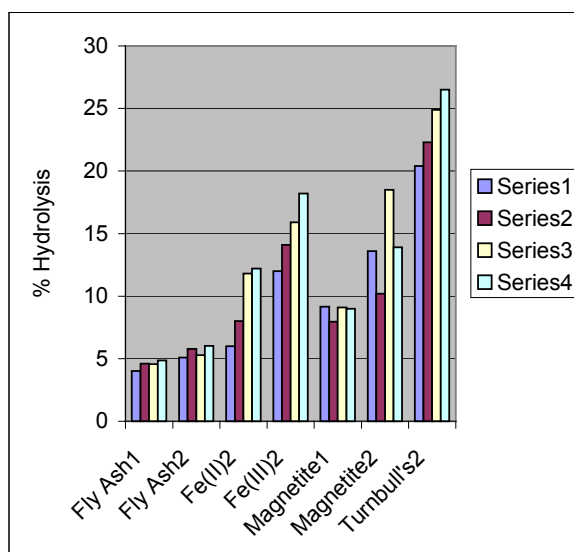
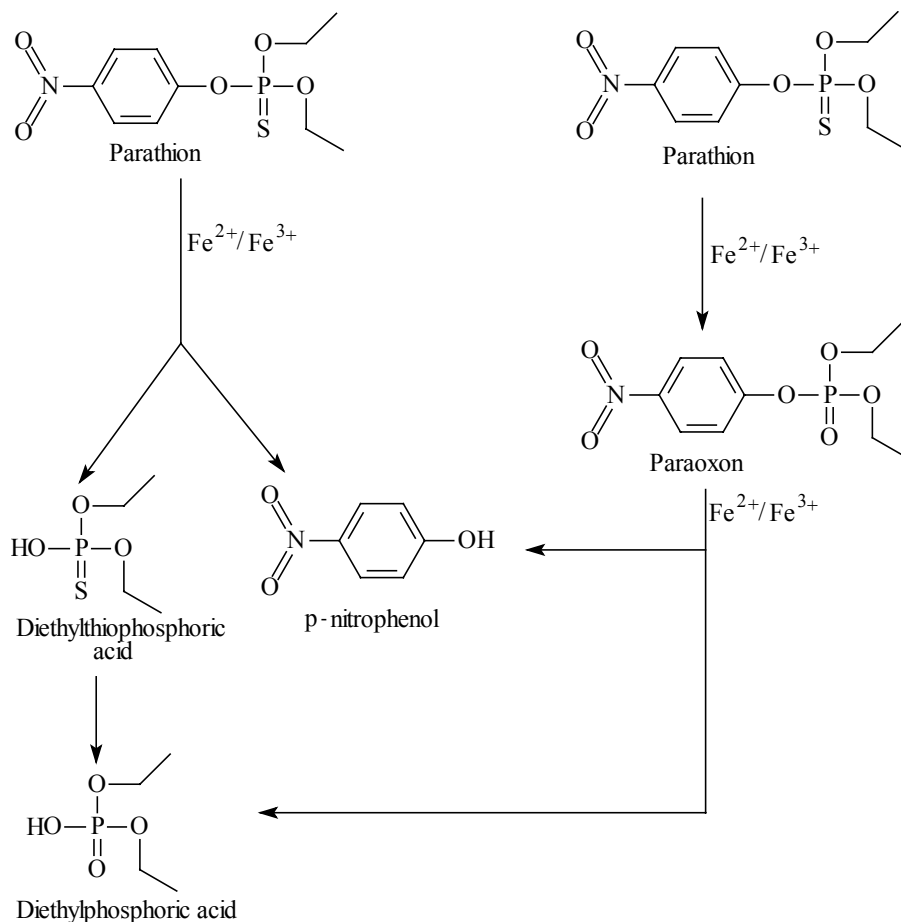


Figure 1. Summary of hydrolysis of 4-nitrophenyl phosphate by several metal ions in aqueous or microemulsion media at ambient temperature, pH 6.5 and 3 min of reaction time. (1 = metal in solid state (as mineral), 2 = metal ions in solution). Series 1 = 1:1 (NPP:metal ratio) in water, Series 2 = 1:1 (NPP:metal ratio) in ME2, Series 3 = 1:2 (NPP:metal ratio) in water, Series 4 = 1:2 (NPP:metal ratio) in ME2.

The physical and chemical properties that make organophosphates effective for pest control also create a potential for environmental contamination. The fate of organophosphate insecticide applied to the soil depends largely on two of its properties: persistence and adsorption. Once applied to a cropland, a pesticide may be taken up by plants, adsorbed to plant surfaces, broken down by sunlight (photodegradation) or ingested by microorganisms, worms, insects and plants making its way up in the food chain. Organophosphates, which adsorb to the soil and immediately incorporate, are protected from photodegradation thereby increasing their persistence and environmental contamination. Organophosphates in the soil break down or degrade over time as a result of several chemical and microbiological reactions. The metabolite of parathion (paraoxon) is responsible for the toxic effects. Generally, chemical reactions result in only partial deactivation whereas soil microorganisms can completely breakdown many pesticides to carbon dioxide, water and other inorganic constituents. Enzymes in the microorganisms are responsible for the degradation reactions and the reactive centers of most of those enzymes contain bimetallic active centers. The bacterial phosphotriesterase catalyzes the hydrolysis of a wide range of organophosphate insecticides and chemical warfare agents. Two strains of bacteria (*Pseudomonas diminuta* and *flavobacterium sp*) were discovered in 1972 that harbored plasmids containing a gene that expressed a protein that acted as an organophosphate hydrolase. The three dimensional X-ray structure of phosphotriesterase has been reported recently [14]. The protein folds as an $(\alpha\beta)_8$ -barrel. The active site is centered on a cluster of six histidine residues. The divalent zinc ions form a binuclear metal center with a bridging hydroxide. Also bridging the binuclear metal center is a carbamate functional group formed from the post-translational modification of lysine-169 with a molecule of CO_2 . The overall protein fold and the structure of the binuclear metal center are very similar to the enzyme urease.



Scheme 1. Alternative degradation pathway of parathion.

The results of our investigation depict a higher degree of hydrolysis for reaction solutions that contained the metal ions in solution than those in the solid form. An enhancement of hydrolysis was also noted in reaction solutions that contained 2:1 metal to nitrophenyl phosphate (NPP) ratio. Addition of the metal ions to NPP at 1:1 molar ratio results in the formation of a monodentate complex which can then undergo intramolecular attack by coordinated water or hydroxide of the metal ion on the phosphorous center, thereby effecting hydrolysis. The formation of four membered chelate ring systems along with ring opened monodentate complexes, which are relatively stable to hydrolysis under the conditions of the experiment have been reported previously [12, 15, 16]. Addition of the second metal ion produces coordination to the available oxygens on the NPP. This will increase the degree of electrophilicity of the phosphorous center thereby making way for the intramolecular nucleophilic attack of the phosphorous center by the coordinated hydroxide or water of the second metal ion.

The increased hydrolysis by magnetite and Turnbull's blue highlights the requirement of binuclear metal complex as the active center. One of the metal ions stabilizes the structure of the metal substrate complex while the second metal plays the reactive role. In biological systems, iron is an essential component of the purple acid phosphatases (PAPs), which are di-nuclear iron

enzymes that catalyze the in vitro hydrolysis of phosphate esters under weakly acidic (or neutral) conditions [17, 18]. The enzyme exists in the reduced form (active); Fe(III)-OH-Fe(II), and oxidized form (inactive); Fe(III)-OH-Fe(III). Iron(II) of the reduced PAP is labile and can be substituted by other divalent metal ions, in particular Zn(II), retaining catalytic activity. Turnbull's blue has an empirical formula $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$, corresponding to a structure having a hexacoordinate low spin Fe(II) bonded through the nitrogen atoms of the cyanide. To achieve this stoichiometry one fourth of the Fe(II) sites are occupied by water molecules. The structure consists of bridging cyanide groups [Fe(II)-CN-Fe(III)] which resembles the reduced (active) form of the purple acid phosphatase. The enhanced hydrolysis achieved by Turnbull's blue seems logical as the complex satisfies the requirement of two metal centers: the structural metal center and the active metal center like most enzymes. Though magnetite (Fe_3O_4) also contains two metal ions per formula unit, its effect of hydrolysis of NPP is less than that of Turnbull's blue. The degree of hydrolysis of NPP by magnetite is comparable to the amount obtained from equimolar mixtures of Fe(II) and Fe(III) with a metal to NPP ratio of 1:2 under the conditions of the experiment. Hence, the presence of two metal centers on a complex seems to play an important role in the effective hydrolysis of phosphate esters.

An increase in hydrolysis was noted for some of the reactions in microemulsion media. Microemulsions are thermodynamically stable transparent dispersions of two immiscible liquids, stabilized by an interfacial film of surfactants [19]. They have the ability to solubilize and disperse the maximum amounts of the reactants and substrates for effective hydrolysis by increasing the surface area of the reaction.

Addition of magnetite powder in the prescribed ratio to the reaction solution of NPP was not found to be as effective as using a solution of magnetite. This is attributed to the sparingly soluble nature of magnetite in aqueous systems. Hence the reactive Fe(II) (aq)/Fe(III)(aq) ions were not available in enough quantity to effect the hydrolysis reaction. It is presumed that the reaction with NPP took place on the surface of the powder where the metal ions prevailed. The hydrolysis of NPP by fly ash was found to be much smaller than the other systems in both the aqueous and solid states. Fly ash is insoluble in water and it was only possible to solubilize fly ash partially by using concentrated hydrochloric acid. We presume that the soluble portions consisted of Fe(II) and Fe(III) ions among others. The hydrolysis observed is the result of those metal ions interacting with the substrate. Fly ash is largely composed of silicates, and its iron content varies from sample to sample. Hence the result obtained seems to correlate with the amount of ferrous and ferric ions present in fly ash.

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