

# Destruction of Organophosphate Agents by Recyclable Catalytic Magnetic Nanoparticles

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## ABSTRACT

Organophosphorus (OP) pesticides and warfare agents are catalytically hydrolyzed in aqueous media by suspensions of magnetite ( $\text{Fe}_3\text{O}_4$ ) nanoparticles modified with poly(1-vinylimidazole-co-acrolein oxime-co-acrylic acid). The oxime- and imidazole-modified magnetite particle serves as a nano-sized particulate carrier with nucleophilic groups immobilized on its surface. The oxime-modified magnetite nanoparticles are colloiddally stable within a wide pH range and are readily recovered for reuse from the aqueous milieu by high-gradient magnetic separation methods with no loss of catalytic activity.

**Keywords:** oxime, imidazole, catalysis, organophosphate agent, poly(1-vinylimidazole-co-acrolein oxime-co-acrylic acid), high gradient magnetic separation.

## 1 INTRODUCTION

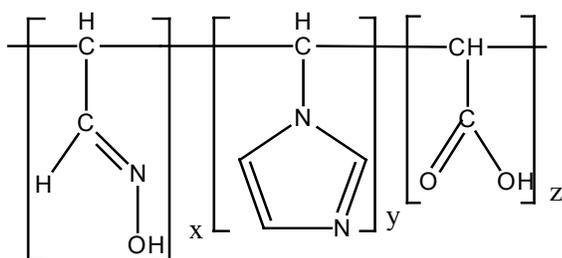
Organophosphates (OPs) are the most widely applied insecticides today. They are used in agriculture, the home, gardens, and veterinary practice. Whilst widely used in agriculture, they are also utilized against household and catering establishment pests, as well as against head lice in humans and a number of ectoparasites in domestic animals. The aerial application of OPs is used to control cereal and vegetable pests. Approximately 40,000 metric tons of various OPs are applied annually to agricultural crops in the U.S. Hence, development of decomposition agents for acutely toxic OP pests as well as warfare agents is of significant utility in both civilian and military fields. Notably, hydrolysis of the P-O bond in the OPs by nucleophilic agents yielding phosphoric acids as the main OP metabolite allows for drastic reduction of the OPs toxicity, because the phosphoric acids are not known to be efficient inhibitors of the acetylcholine esterase. We have recently discovered a group of catalytic decomposition agents, friendly to the environment and inexpensive, which could be readily dispersed in an aqueous medium, be it an aqueous reservoir or bodily fluid, maintain their ability to decompose OP at neutral pH, and be capable of being efficiently removed from the medium by a high-gradient magnetic separation (HG-MS)[1]. Such agents comprised magnetite ( $\text{Fe}_3\text{O}_4$ ) nanoparticles modified by a powerful  $\alpha$ -nucleophile, oxime group, as in a common antidote, 2-pralidoxime (PAM), or its polymeric analog, poly(4-vinylpyridine-N-phenacyloxime-co-acrylic acid). In the

present work, we adopted analogous approach toward the OP decomposition agents, but designed a novel copolymer acting as both an efficient nucleophilic agent on the magnetite surface as well as a stabilizing agent enabling an aqueous stability of magnetite suspensions in a broad range of pH. The presence of the oxime group in the novel copolymer was achieved by the use of acrolein that is readily converted to the acrolein oxime by oximation with hydroxylamine. However, the use of polyacrolein, per se a water- and common solvents-insoluble polymer, was impossible for our intended application. Therefore, we utilized a seeded copolymerization of acrolein with an amphiphilic copolymer of 1-vinylimidazole (VIm) and acrylic acid (AA), to arrive at an acrolein copolymer that was subsequently oximated. An analogous free-radical polymerizations of acrolein in basic aqueous conditions initiated by persulfates and using polypyrrole as a seed agent has been reported previously [2]. Application of VIm has been rationalized in terms of imidazole's well-known nucleophilicity that could be weaker than the oxime's, but still sufficient to catalyze ester hydrolysis in the enzyme-like fashion [3]. In organophosphate-hydrolytic enzymes, histidine imidazole serves as a base, deprotonating a water molecule and generating the attacking hydroxide ion that produces the hydrolysis [4]. Furthermore, vinylimidazole is hydrophilic even at pH above its  $\text{pK}_a$  and poly(1-vinylimidazole) has been shown to maintain colloidal stability of magnetic iron oxide particle up to pH 10 [5]. Ion-exchange equilibria and complexation between the positively charged imidazole-modified particles and organophosphoric acids resulting from the OP hydrolysis can contribute to the acid metabolite recovery along with the particles, using HGMS. Finally, AA was chosen as a minor component of the copolymer to further stabilize magnetite particle at neutral pH. The resulting copolymer was capable of stabilizing magnetite nanoparticles, which catalyzed hydrolysis of a model OP nerve agent, diisopropyl fluorophosphate (DFP), and could be recovered from aqueous media by the HGMS, as described below.

## 2 EXPERIMENTAL

2.1 *Polymer Synthesis:* Copolymer of N-vinylimidazole (VIm), acrolein (Ac), and acrylic acid (AA) [p(VIm-AcOx-AA)] was synthesized by seeded, free-radical copolymerization using potassium persulfate as an initiator, followed by oximation. A three-necked flask containing a

deoxygenated solution of 0.1 mol (9.9 mL) of VIm, 0.025 mol (1.82 mL) of AA, and 15 mg of  $K_2S_2O_8$  in deionized water (50 mL) was deaerated by nitrogen purge and kept at 70 °C for another 0.5 h. Then 0.1 mol (5.9 mL) of Ac were added to the reaction mixture via a syringe, followed by addition of 10 mg/mL aqueous  $K_2S_2O_8$  solution (10 mL) and stirring. The reaction mixture was kept under nitrogen blanket at 70°C overnight. The resulting black, viscous copolymer [p(VIm-Ac-AA)] solution was repeatedly washed by acetone and freeze-dried. The aldehyde groups of the acrolein in the copolymer were converted to acrolein oxime by reacting the p(VIm-Ac-AA) copolymer (15 g) with 200 mL of a freshly prepared anhydrous methanol solution containing 0.15 mol (10.4 g) of hydroxylamine hydrochloride and 0.15 mol (6 g) of sodium hydroxide. The methanolic solution of the polymer and hydroxylamine was kept under reflux while stirring at 70°C for 2 days and then methanol was evaporated and the resulting solids were repeatedly washed with excess water and acetone on a paper filter. The resulting polymer was exhaustively dialyzed against 10 mM aqueous  $H_2SO_4$  solution using a Spectra/Por® membrane (MWCO, 3.5 kDa). The purified p(VIm-AcOx-AA) samples were lyophilized and stored dry at -20 °C until further use.  $(C_{37}H_{54}N_{12}O_6)_x$ , found (calc): C 58.14 (58.25); H 7.61 (7.13); N 22.39 (22.03).  $^1H$  NMR (400 MHz,  $CD_3OD$ ): d 1.6 (m, 2 H,  $CH_2$ - in the main chain), 2.2, 3.5 (m, 1 H, CH- in the main chain), 7.25 (m, 2H, imidazole), 7.36 (s, 1H, oxime), 7.7 (m, 1H, imidazole). FTIR (KBr): 3370 (bonded N-H, OH stretch), 2918, 2850 (N-H...N= stretch), 1695 (C=O), 1670 (aldoxime C=N), 1650 (aldoxime C=N), 1560, 1435, 1371, 1266, 1094, 938, 732  $cm^{-1}$ . Weight-average MW by GPC in N,N-dimethylacetamide was 128.5 kDa, polydispersity index 1.82. The structure of the p(VIm-AcOx-AA) copolymer is depicted in Scheme 1.



$$x : y : z = 4 : 4 : 1$$

Scheme 1. p(VIm-AcOx-AA) copolymer.

**2.2. Particle Synthesis:** Magnetic nanoparticles were produced by chemical coprecipitation of iron(II) and iron (III) chlorides. Namely, 1.88 g (7.0 mmol) of  $FeCl_3 \cdot 6H_2O$  and 0.69 g (3.5 mmol) of  $FeCl_2 \cdot 4H_2O$  were added to 40 mL of deionized water and the solution was deaerated by nitrogen purge in a stirred 250-mL three-necked flask and

temperature of the flask contents was brought to 80 °C. An aqueous/methanol (1:1 v/v) solution of the p(VIm-AcOx-AA) copolymer (6.25 wt%, 40 mL total, pH adjusted to 6) was added to the flask and the resulting mixture was equilibrated at 80 °C while stirring under nitrogen purge. Then the nitrogen purge was ceased and the contents of the flask were at once added to 80 mL of a 28% ammonium hydroxide and the mixture was vigorously stirred for 10 min. The resulting precipitate possessed strong magnetic properties and was thus separated from the liquid by decantation using a magnetic separator. The precipitate was dried and resuspended in deionized water [1]. The suspension was dialyzed against excess deionized water (membrane MW cut-off, 3.5 kDa) and lyophilized. The composition of the oxime-containing particles were assessed by elemental analysis as follows.  $[(C_{37}H_{54}N_{12}O_{14}(Fe_3O_4)_2)_x]$ , found (calc): C 35.75 (36.25); H 4.09 (4.44); Fe 27.12 (27.33); N 13.74 (13.71), oxime group content 3.2 mmol/g.

## 2.2 Kinetic measurements

Kinetics of the DFP decomposition were measured at 25 °C with an Orion 96-09 combination fluoride electrode (Thermo Electron Corp.) and a Model 45 Dual Display Multimeter (Fluke Corp.) connected to a PC with FlukeView Forms software for data processing. The electrode was immersed in a stirred 9-mL aqueous sample and the electrode potential-time output was recorded continuously. No significant changes in pH, set initially at 7.0, were observed in any of the runs. The electrode was calibrated in independent series of experiments using aqueous solutions of sodium fluoride.

## 2.3 Magnetic Separation and Reuse

High-gradient magnetic separation (HGMS) experiments were performed using a cylindrical plastic column with an internal diameter of 7 mm and a length of 20 cm packed with 3.6 g of type 430 fine-grade stainless steel wool (40-66 mm diameter) placed inside a quadrupole magnet system comprising four nickel-plated Neodymium Iron Boron 40 MGOe permanent magnets sized 18x1.8x1.8 cm each (Dura Magnetics, Inc.). The flux density generated inside the packed column was ca. 0.73 Tesla.

Magnetic washing of the particles was performed as described previously [1]. The resulting suspension was subjected to the kinetic experiment using electrode detection of the fluoride ions generated by the DFL decomposition. The process of particle recovery and reuse was repeated in two sequential cycles.

## 3 RESULTS AND DISCUSSION

Effect of pH on electrokinetic mobility and particle size of the magnetite modified with the p(VIm-AcOx-AA) copolymer is shown in Fig.1. As is seen, the particles

remained stable, with the number-average hydrodynamic diameter varying in the range 125-150 nm. No particle precipitation and sedimentation was observed up to pH 11.5. The  $\zeta$ -potential was significant and positive at pH<8, reflecting the positive charge on imidazole moieties, and turned negative above pH 8, when the contribution from the negatively charged carboxyls of AA turned entire particle negative as the imidazole moieties were no longer charged at these pH. The  $pK_a$  of the poly(1-vinylimidazole) is 4.9 [6], and the pI of the VIm and AA copolymers (VIm: AA mol ratio 4:1) has been reported at 6.5 [7]. Attachment of the poly(1-vinylimidazole) to maghemite particles resulted in the particles with pI 7.4 [5], reflecting a shift to higher pH required for the ionization of the immobilized imidazole. Even larger shift to pH  $\geq 8$  is observed herein.

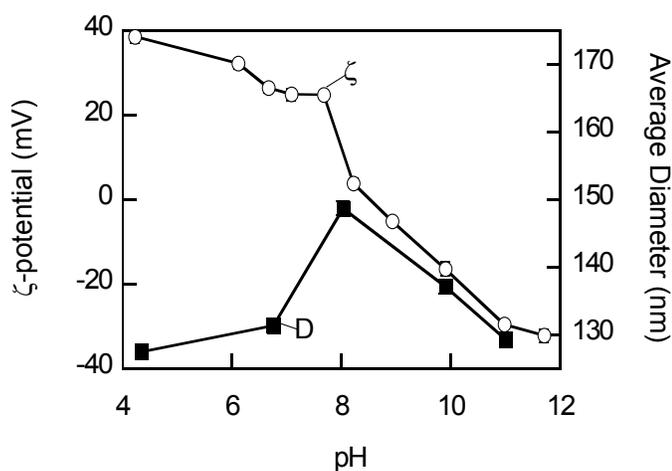


Figure 1: Effect of pH on  $\zeta$ -potential and hydrodynamic diameter of magnetite particles modified with p(VIm-AcOx-AA). T=25°C; 10 mM Tris buffer with pH adjusted by 1 M NaOH or HCl.

Hydrolysis of DFP to produce the fluoride ion was monitored by the ion-selective fluoride electrode. Typical electrode response curves are shown in Fig.2.

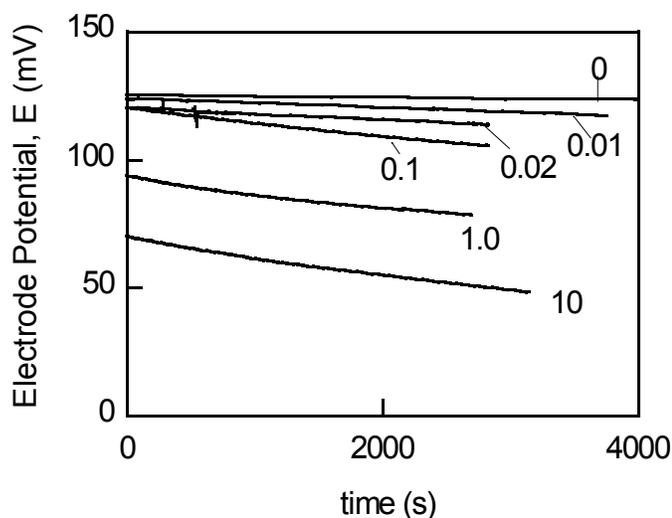


Figure 2: Potential-time response of fluoride-selective electrode to 0.6 mM DFP concentration in the presence of varying concentrations of magnetite particles modified with p(VIm-AcOx-AA). T=25°C; 10 mM Tris, pH 7.0. Numbers stand for particle concentrations in mg/mL. Addition of DFP to a suspension of oxime-containing particles, resulted in the rapid appearance and accumulation of the fluoride ions, as is seen from the response of the ion-selective electrode. The electrode potential was converted to the time-dependent fluoride concentration ( $C_t$ ) readings, using electrode calibration curves in sodium fluoride solutions [1]. The initial slope of the  $C_t$  vs  $t$  kinetic curves corresponds to the initial rate of the DFP hydrolysis ( $v_o$ ). The observed rate constant for the DFP hydrolysis ( $k_{obs}$ ) was obtained from the experimental data:  $-\ln(1 - C_t/[DFP]_o) = k_{obs}t$ , where  $[DFP]_o$  is the initial concentration of the substrate. Fig.3 shows typical kinetic curves in terms of the above equation. It further illustrates the particle recovery results.

Recovery of the nanoparticles from the aqueous solutions was achieved in a series of magnetic filtration experiments in each of which, following the successful catalytic hydrolysis of DFP by the oxime-coated nanoparticles, the suspension of particles was passed through the HGMS filter placed inside the magnet device. The magnetic particles were trapped in the filter and subsequently recovered by removing the steel wool-packed column from the magnetic environment and passing fresh water through the filter; these particles were then reused in the hydrolysis of a fresh batch of DFP solution (Fig.3). This cycle of the DFP hydrolysis and particle filtration and collection was repeated twice. As is seen in Fig.4, essentially complete recovery and reuse of the particles was possible. The  $k_{obs}$  constants for DFP hydrolysis were determined in three cycles to be unchanged at  $(2.6 \pm 0.07) \times 10^{-5}$ .

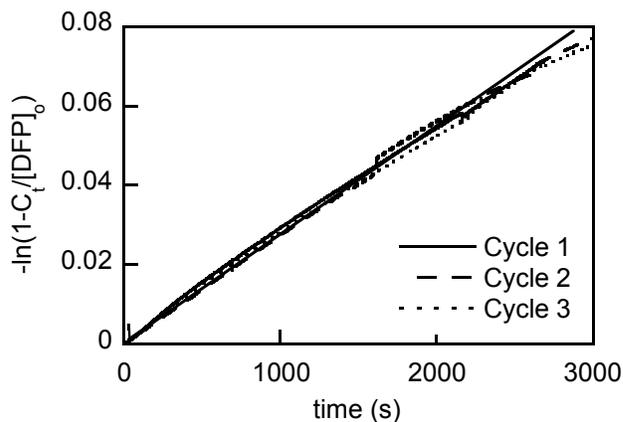


Figure 3: Catalytic stability of magnetic nanoparticles modified by p(VIm-AcOx-AA) indicated by essentially unchanged kinetic profiles observed for DFP hydrolysis

when the particles are recovered, washed, and reused. T=25°C, pH 7.0, 10 mM Tris; initial DFP concentration in all cycles, 0.6 mM. Cycle 1 denoted the first use of the particles, Cycle 2 the first magnetic recovery and resuspension of the particles at 1 mg/mL, and Cycle 3 the second magnetic recovery and resuspension of the particles at 1 mg/mL. The straight line illustrates the average slope of the kinetic curves used to calculate the  $k_{\text{obs}}$ .

The observed rate of DFP hydrolysis in 10 mM Tris buffer at pH 7.0 in the presence of particles was markedly higher than the  $k_{\text{obs}}$  of the spontaneous hydrolysis, even at very small particles concentrations (Fig.4). The apparent second-order hydrolysis rate constant,  $k'' = v_o / [Catalyst]_o [Substrate]_o$  with p(VIm-AcOx-AA)-modified magnetite was 2-5 times higher than with the previously reported [1] magnetite modified with poly(4-vinylpyridine-N-phenacyloxime-co-acrylic acid), at similar initial oxime and substrate concentrations.

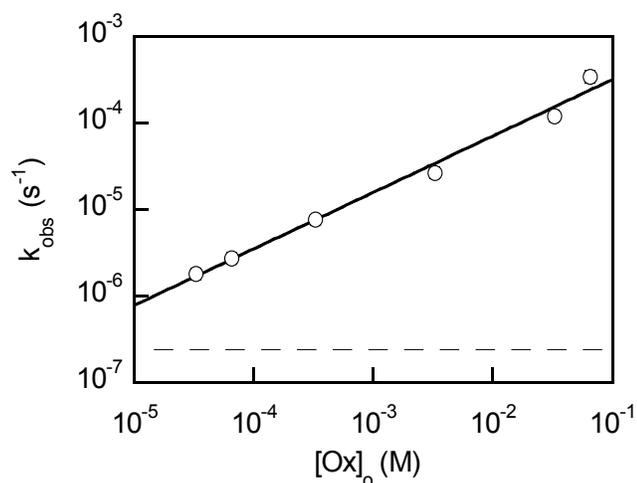


Figure 4: Dependence of the observed DFP hydrolysis kinetics constants ( $k_{\text{obs}}$ ) on the effective initial concentration of the oxime groups ( $[\text{Ox}]_o$ ) in suspensions of magnetite modified with p(VIm-AcOx-AA). T=25°C; 10 mM Tris, pH 7.0,  $[\text{DFP}]_o=0.6$  mM. Broken line shows  $k_{\text{obs}}$  for the spontaneous hydrolysis of DFP in the buffer solution alone.

## 4 CONCLUSIONS

Novel, recyclable oxime- and imidazole-modified magnetite nanoparticles have been reported. The particles of hydrodynamic diameter of 125-150 nm are stable in aqueous suspensions in a wide range of pH, catalyze the hydrolysis of the OPs and can be recovered by high gradient magnetic separation and reused without a significant loss of hydrolytic activity. Studies are underway to clarify whether the recovery of the particles can also

reduce the concentration of the phosphoric acids (the OP metabolites), from water.

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