

Synergistic Catalysis of Dimetilan Hydrolysis by Metal Ions and Organic Ligands

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Hydrolysis of the insecticide dimetilan, which possesses a *N,N*-dimethylcarbamate moiety and a *N,N*-disubstituted urea moiety, has been used to explore metal ion-organic ligand synergistic effects on the degradation of agrochemicals. Dimetilan hydrolysis is strongly catalyzed by +II transition metal ions exhibiting strong affinities for nitrogen- and oxygen-donor ligands (Ni^{II} , Cu^{II} , and Zn^{II}) but not Pb^{II} , which exhibits only a weak affinity. Comparisons among these four metal ions strongly suggest that metal ion coordination of dimetilan is necessary for catalysis to occur. A combined mechanism of metal ion coordination of dimetilan and the generation of a MeOH^+ nucleophile is most plausible for the metal-catalyzed hydrolysis. In the absence of metal ions, citric acid (CIT), ethylenediamine (EN), and *N*-(2-hydroxyethyl)ethylenediamine (HEEN) ligands alone do not affect dimetilan hydrolysis. However, addition of these ligands significantly changes the catalytic effect of metal ions. CIT and EN reduce the capability of free metal ions to catalyze agrochemical hydrolysis. In contrast, HEEN enhances the catalytic effect of metal ions on dimetilan hydrolysis. The hydroxyl group of HEEN is believed to facilitate metal ion catalysis by acting as an intramolecular nucleophile or general base catalyst within the dimetilan-metal ion-HEEN ternary complex. This study provides a few examples of many possible synergistic/cooperative effects of metal ions and organic ligands in the environment often demonstrated in biological systems such as extracellular enzymes.

Introduction

Hydrolysis is believed to be the predominant degradation pathway for a number of important agrochemicals under field conditions. Hydrolysis rates measured in the laboratory, however, often differ significantly from rates measured in field test plots. This finding leads to the hypothesis that simple aqueous media commonly employed in the laboratory (e.g., distilled water plus the agrochemical with a pH buffer) lack chemical or biological constituents that catalyze or inhibit hydrolysis under field conditions. As far as chemical constituents are concerned, single-amendment experiments have shown that dissolved metal ions (1), simple hydrous metal oxides (2–5), and clays (6, 7) can increase hydrolysis rates of agrochemicals dramatically. Catalysis may arise from reaction of the added chemical constituent with the agro-

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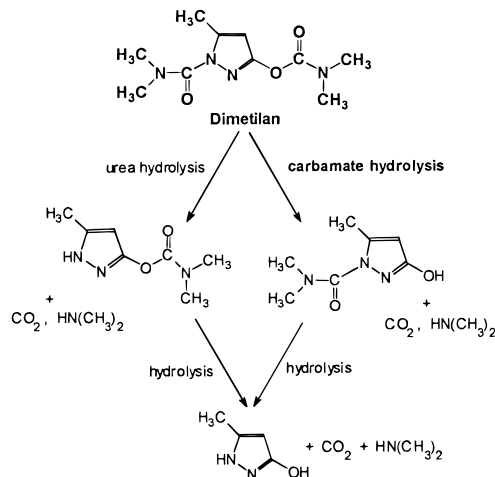


FIGURE 1. The hydrolysis pathways of dimetilan.

chemical or with the attacking nucleophile (e.g., H_2O or OH^-). In other situations, dissolved metal ions and metal oxides decrease the hydrolysis rates of agrochemicals (8, 9).

Synergistic effects, which have received little attention in the past, can be addressed using dual-amendment experiments. Simultaneous addition of a metal ion and an organic ligand results in the formation of metal–ligand complexes which may react in ways quite distinct from the free metal ion or free ligand. The importance of such synergistic effects depends on the concentrations of metal–ligand complexes in environmental media and their inherent reactivity.

Dissolved metal ions in aqueous environmental media have been discussed recently (1, 8). As far as organic ligands are concerned, appreciable concentrations of low-molecular weight organic acids (e.g., oxalic acid and citric acid (10)), monosaccharides (e.g., glucose and fructose (11)) and sugar acids have been detected in soil waters and surface waters. Hydroxamate siderophores (12) and aminocarboxylate siderophores (e.g., mugineic acid (13) and rhizoferrin (14, 15)) are produced by organisms as diverse as bacteria, fungi, and plants to coordinate iron(III). Other organism-derived organic ligands and metal-organic ligand complexes, including porphyrins and hydrolytic enzymes, have also been identified in soils and waters (16–18). The identified extracellular hydrolytic enzymes include protease, esterase, phosphatase, and urease.

Natural chemical and biological processes continually rework the organic carbon pool, generating new organic chemicals with distinctive properties. The higher molecular weight, polyelectrolyte fraction is referred to as humic substances. Hydrolysis in the presence of humic substances has been studied in the absence of added metal ions (19); the effects were found slight.

In this work, hydrolysis of the insecticide dimetilan (Figure 1) is used to explore metal ion-organic ligand synergistic effects. In common with early carbamate insecticides such as pirimicarb, dimetilan possesses a *N,N*-dimethylcarbamate moiety that inhibits cholinesterase enzymes (20). Hydrolysis can occur via nucleophilic attack at this carbamate group or at the substituted urea group (Figure 1). Four divalent metal ions, Ni^{II} , Cu^{II} , Zn^{II} , and Pb^{II} , are included in this study. Although these metal ions are probably not found at high concentrations in most environments except in certain local situations, the four metal ions possess distinctive chemical properties that provide insight regarding mechanisms of catalysis. Note that copper ranks third in fungicide use in the

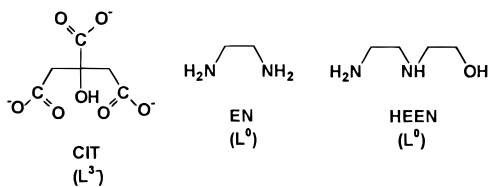


FIGURE 2. Structure of citric acid (CIT), ethylenediamine (EN), and *N*-(2-hydroxyethyl)ethylenediamine (HEEN) ligands.

U.S. (21), and zinc is moderately abundant in the environment and in biological systems. Citrate (CIT) (Figure 2) serves as a representative carboxylate-based naturally occurring organic ligand. Experiments with the synthetic organic ligands ethylenediamine (EN) and *N*-(2-hydroxyethyl)ethylenediamine (HEEN) (Figure 2) highlight the interrelationships between coordination chemistry and catalysis.

Mechanistic Foundations. As discussed in a number of recent publications (1, 8), metal ions catalyze hydrolysis via one or a combination of the following three generalized mechanisms: **Mechanism 1:** The metal ion coordinates the substrate in a manner that raises its susceptibility toward nucleophilic attack. **Mechanism 2:** The metal ion coordinates the nucleophile in a manner that raises its reactivity toward electrophilic sites. **Mechanism 3:** The metal ion coordinates the leaving group, facilitating its exit from the higher-coordinate intermediate. It should be noted that **Mechanism 2** has been strongly tied to the ability of metal ions to induce the deprotonation of coordinated water through reactions such as $\text{Me}^{2+} + \text{H}_2\text{O} = \text{MeOH}^+ + \text{H}^+$, $^*K_1 = [\text{MeOH}^+][\text{H}^+]/[\text{Me}^{2+}]$. Cu^{2+} and Pb^{2+} (possessing \log^*K_1 values of -7.5 and -7.6 , respectively) are far better at inducing water deprotonation than Ni^{2+} and Zn^{2+} (\log^*K_1 values of -9.9 and -9.0) (22).

Organic ligand catalysis on hydrolysis is believed to occur through one or a combination of two different mechanisms: **Mechanism 4:** General acid–base catalysis, in which the organic ligand donates a proton to the substrate or accepts a proton from the attacking nucleophile in a manner that accelerates the reaction. **Mechanism 5:** Nucleophilic catalysis, in which the organic ligand serves as a nucleophile, forming an adduct which rapidly hydrolyzes into the expected hydrolysis products.

Organic ligands cannot coordinate the substrate or nucleophile, except through very weak hydrogen-bondings. Hence, synergistic effects necessarily involve the formation of metal–ligand complexes and arise from their effects on **Mechanisms 1–5**. The predominant coordination number of the divalent metal ions included in this study is either five (for Cu^{II}) or six (for Ni^{II} , Zn^{II} , and Pb^{II}). Whether or not the central metal ion can simultaneously coordinate two entities (e.g., organic ligand plus substrate; organic ligand plus nucleophile) has important mechanistic consequences.

If simultaneous coordination is not possible, metal–ligand complex formation prevents either the metal ion or the organic ligand from participating in the hydrolysis reaction. As a consequence, catalysis by any of the five mechanisms is impeded. A number of studies, for example, have shown that metal ions lose their catalytic properties when coordinated to carboxylate-containing ligands (23, 24) and to bipyridine (25).

When simultaneous coordination is possible, reactivity within the **ternary complexes** that result must be considered. Positive synergistic effects of this kind are believed to be responsible for the high catalytic activity of metalloenzymes (26). Chemists have sought simpler, biomimetic complexes built from hydroxyl-, carboxylate-, amino-, and thiol-containing ligands in order to explore these effects (e.g., refs 27 and 28).

Coordination of the central metal ion by an organic ligand can alter the tendency of any remaining coordinated water molecules to undergo deprotonation. Macrocyclic polyamines, for example, have been observed to influence catalysis via **Mechanism 2** by raising or lowering the \log^*K_1 associated with the $\text{Me}^{2+} + \text{H}_2\text{O} = \text{MeOH}^+ + \text{H}^+$ reaction (29, 30).

Hydroxyl groups acting in concert with metal ions have been shown to participate in synergistic effects on hydrolysis (31, 32) and transesterification (33, 34) reactions. The hydroxyl group (analogous to serine within metalloenzymes) undergoes metal ion-assisted deprotonation and subsequently serves as an intramolecular nucleophile within a metal ion–ligand–substrate ternary complex. As the reaction occurs, the central metal ion holds the substrate and nucleophile together and hence serves as a template. The organic ligand *N*-(2-hydroxyethyl)ethylenediamine (HEEN) was designed with this mechanism in mind; two amine groups anchor the ligand onto the central metal ion, and the pendant hydroxyl group is close enough to serve as an intramolecular nucleophile. Using *p*-nitrophenyl picolinate as the hydrolyzable substrate and Zn^{II} as the central metal ion, a dramatic synergistic effect can be observed (33). As far as other functional groups are concerned, pendant carboxylate groups have been observed to facilitate metal ion-catalyzed amide hydrolysis (35), and pendant amine groups have been reported to participate in other reactions as general acid catalysts (36).

Materials and Methods

Chemical Reagents. Reagent grade water (18 M Ω -cm resistivity) was prepared using a glass distillation apparatus and a Milli-Q reagent-grade water system (Millipore, Bedford, MA). The inorganic reagents $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, ZnCl_2 , and NaCl were purchased from Aldrich (Milwaukee, WI) at the highest possible purity. Acetic acid, NaOH (from J. T. Baker, Phillipsburg, NJ), and MOPS (4-morpholinepropanesulfonic acid, from Aldrich) were employed as pH buffers. Dimetilan (1-dimethylcarbamoyl-5-methylpyrazol-3-yl-dimethylcarbamate) at greater than 98% purity was obtained from the EPA Pesticides Repository and used without further purification. Citric acid (CIT), ethylenediamine (EN) (from Aldrich), and *N*-(2-hydroxyethyl)ethylenediamine (HEEN) (from Lancaster, Windham, NH) at greater than 99% of purity were used without further purification.

Hydrolysis Experiments. All glassware was soaked in 6 N HNO_3 and rinsed several times with reagent-grade water prior to use. The brown glass amber vials were autoclaved prior to use, and reaction solutions were filter-sterilized to inhibit biotic reactions. At 5–20 mM concentrations, acetic acid with NaOH buffer was used for pH 4.0 to 5.7, and MOPS with NaOH buffer was used for pH 5.9 to 8.0. Reaction solutions were initially prepared with buffer, metal salt, and organic ligands. Solution pH was adjusted by adding very small amount of strong acid (HCl) or base (NaOH). After 3 to 4 h of stirring, an appropriate amount of freshly prepared dimetilan stock solution was added to initiate hydrolysis. Reaction solutions were continuously stirred in a 25 °C water bath, and aliquots of solution were periodically collected for analysis.

Decreases in the concentration of dimetilan were monitored using a reversed-phase HPLC with a μ -Bondapac- C_{18} column and a UV detector set at 230 nm (Waters, Milford, MA). The employed eluent consisted of 30% acetonitrile and 70% 1.0 mM phosphate (pH 7.0) buffer solution. Plots of the log of dimetilan concentration versus time were linear, indicating pseudo-first-order kinetics. Hydrolysis rate constants k_{obs} (in hours $^{-1}$) reported in this work correspond to the slopes of these plots obtained by least squares linear regression (r^2 between 0.980 and 0.999). If the hydrolysis

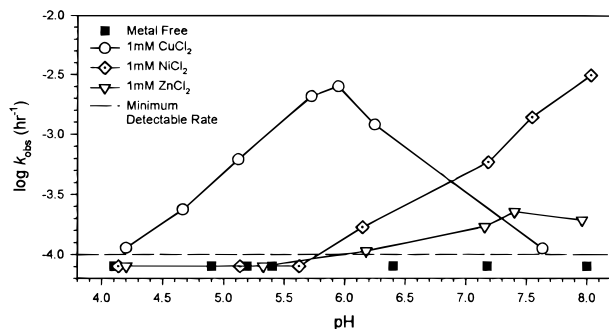


FIGURE 3. Effect of pH on metal ion-catalyzed hydrolysis of dimetilan. Reaction conditions: 5.98×10^{-5} M dimetilan, 1.0 mM CuCl_2 , NiCl_2 , or ZnCl_2 , 6.67 mM acetate (up to pH 5.7) or MOPS (pH 5.9 and higher) buffer, 25 °C.

TABLE 1. Stability Constants for Metal Ion-Ligand Complexes (22)^a

		log K of Cu-L _n		log K of Zn-L _n	
EN	pK_{a1} :	6.85	10.50 (CuL)	5.66	(ZnL)
(L ⁰)	pK_{a2} :	10.93	19.60 (CuL ₂)	10.60	(ZnL ₂)
HEEN	pK_{a1} :	6.34	10.02 (CuL)	5.28	(ZnL)
(L ⁰)	pK_{a2} :	9.56	17.45 (CuL ₂)	10.07	(ZnL ₂)
CIT	pK_{a1} :	3.13	3.19 (CuHL)	4.79	(ZnL)
(L ³⁻)	pK_{a2} :	4.76	14.80 (CuL ₂)	2.78	(ZnHL)
	pK_{a3} :	6.40		1.30	(ZnHL ₂)

^a For the reactions: $\text{Me}^{2+} + x\text{H}^+ + y\text{L}^{n-} = \text{MeH}_x\text{L}_y^{(2+x-ny)+}$.

reaction was too slow, decreases in the concentration of dimetilan could not be distinguished from the experimental error of the HPLC techniques. For this reason, k_{obs} values below 1.0×10^{-4} hours⁻¹ cannot be measured.

Results and Discussion

Metal Ion-Free Solutions. Dimetilan hydrolysis experiments in the absence of metal catalysts were performed at 25 °C in solutions buffered between pH 4.0 and 8.0 (Figure 3). No loss of dimetilan was detected after 14 days of reaction. In a separate set of experiments, 0.1 μM to 0.1 M of EN, HEEN, and CIT ligands were added at pH 5 and 7. No loss of dimetilan was detected even when 0.1 M concentrations of ligands were added. Hence, k_{obs} values were below 1.0×10^{-4} hours⁻¹ in metal ion-free solutions and cannot be quantified.

The lack of observable dimetilan hydrolysis in metal ion-free and organic ligand-free solutions is quite reasonable. The *N,N*-dimethylcarbamate moiety is expected to be more susceptible toward hydrolytic attack than the *N,N*-disubstituted urea moiety; oxygen is more electronegative than nitrogen and less able to participate in resonance stabilization with the carbonyl group. Uncatalyzed hydrolysis of the *N,N*-dimethylcarbamate moiety has been estimated to occur on time scales of hundreds of years (37).

Mechanisms 4–5 postulated for organic ligand catalysis are apparently not operative here. The pK_a of the hydroxyl group of HEEN has been reported to be near 12 (33), comparable to the reported pK_a (11.6) of the hydroxyl group of CIT (38). Although the corresponding alcoholate ions are undoubtedly strong nucleophiles, they are too strongly protonated to have any significant effect on hydrolysis. The pK_a (Table 1) values for the carboxylate groups of CIT (3.13, 4.76, 6.40) and for the amine groups of EN (6.85, 10.93) and HEEN (6.34, 9.56) are close enough to solution pHs that general acid–base catalysis should be possible, if an appropriate mechanism was available.

Metal Ion Catalysis. Experiments with divalent metal ions employed the same temperature, pH range, and duration as the experiments performed in metal ion-free solutions. In

the presence of metal ions, loss of dimetilan followed pseudo-first-order kinetics.

As shown in Figure 3, in the presence of 1.0 mM Cu^{II} , measurable values of $\log k_{\text{obs}}$ can be obtained at pH 4.2. As the pH is increased to 5.95, $\log k_{\text{obs}}$ increases linearly with slope equal to 0.80. Above this pH, k_{obs} decreases dramatically and approaches minimum measurable values at pH 7.6. In this pH region, solutions became cloudy as Cu^{II} precipitated.

In the presence of 1.0 mM of Ni^{II} , the pH must be 1.6 log units higher than observed with Cu^{II} in order to obtain measurable values of $\log k_{\text{obs}}$. Again, $\log k_{\text{obs}}$ increases linearly as the pH is increased (Figure 3). The slope is equal to 0.67, slightly less than the slope observed with Cu^{II} . Ni^{II} -containing solutions remained clear throughout the pH range examined, and no drop off in k_{obs} at high pH was observed. For this reason, k_{obs} is actually higher than observed with Cu^{II} when the pH is greater than 7.18.

1.0 mM Zn^{II} is a much less effective catalyst than Cu^{II} and Ni^{II} , and measurable k_{obs} values could only be obtained at above pH 6.18. For the first three data points, $\log k_{\text{obs}}$ increased with increasing pH, yielding a slope equal to 0.25, much lower than observed with Cu^{II} and Ni^{II} . At pH 7.96, k_{obs} decreased, and the solution became cloudy as Zn^{II} precipitated.

1.0 mM Pb^{II} did not yield measurable values of k_{obs} throughout the pH range examined ($4.0 < \text{pH} < 7.4$). Solutions became cloudy at above pH 6.0 as Pb^{II} precipitated.

The speciation of 1.0 mM of Ni^{II} , Cu^{II} , Zn^{II} , and Pb^{II} as a function of pH under the conditions employed in these experiments was calculated by the equilibrium computer program HYDRAQL (39) using equilibrium constants from the CRITICAL database (22). The four divalent metal ions begin to precipitate as $\text{Me}(\text{OH})_{2(\text{s})}$ solids as the pH is increased in the following order: Pb^{II} (pH 5.8) < Cu^{II} (pH 6.2) < Zn^{II} (pH 7.3) < Ni^{II} (pH 8.2). Therefore, Pb^{II} has the lowest and Ni^{II} has the highest solubility. The appearance of turbidity, which we interpret as precipitation, is consistent with these trends in solubility. Dramatic decreases in k_{obs} that correlate with the occurrence of precipitation indicate that dissolved metal ions are much more reactive catalysts than metal (hydr)oxide surfaces.

Solution pH affects the speciation of metal ions, thereby affecting their interaction and reactivity toward substrates. The reported k_{obs} represents the overall rate for the metal-catalyzed hydrolysis of dimetilan and can be expressed as a function of different metal species in the solution

$$k_{\text{obs}} = k_{\text{Me}^{2+}}[\text{Me}^{2+}] + k_{\text{MeOH}^+}[\text{MeOH}^+] + k_{\text{MeAc}^+}[\text{MeAc}^+] + k_{\text{Me}(\text{Ac})_2^0}[\text{Me}(\text{Ac})_2^0] + k_{\text{MeCl}^+}[\text{MeCl}^+] + \dots \text{etc.} \quad (1)$$

where Ac is the acetic acid buffer and k_i is the rate constant for the metal species *i*. Under the experimental conditions, Me^{2+} , MeOH^+ , MeAc^+ , $\text{Me}(\text{Ac})_2^0$, and MeCl^+ species are present at concentrations considerably higher than other species such as MeCl_2^0 , $\text{Me}(\text{OH})_{2(\text{aq})}$, $\text{Me}(\text{OH})_3^-$, $\text{Me}(\text{OH})_4^{2-}$, etc. Determination of the rate constant for each metal species is rather difficult; however, the relative importance of each metal species to the overall rate can be discerned through careful experiments. For instance, varying acetate and chloride concentrations had negligible effects on the rate of metal-catalyzed hydrolysis of dimetilan (data not shown), indicating that the acetate- and chloride-coordinated metal species are unlikely to play a major role in determining the rates of dimetilan hydrolysis.

Metal-to-metal comparisons can be used to obtain information regarding mechanism. For *N,N*-dialkylcarbamates and *N,N*-disubstituted ureas, nucleophilic attack is usually the rate-limiting step, rather than breakdown of a

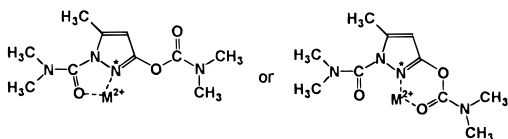


FIGURE 4. Metal ions can coordinate dimetilan through either a five- or a six-membered chelate ring.

higher-coordinate intermediate. Hence, **Mechanism 3** can be excluded. As noted in an earlier section, \log^*K_1 comparisons indicate that Cu^{II} and Pb^{II} possess nearly the same ability to induce the deprotonation of coordinated water, while Ni^{II} and Zn^{II} have much less ability. The observation that Pb^{II} does not yield significant catalysis strongly suggests that deprotonation of coordinated water, and hence **Mechanism 2**, is not the sole mechanism. It is possible, however, for **Mechanism 2** to be operative in concert with **Mechanism 1**. Such a “push-pull” mechanism has often been suggested (40–43). The fact that, within the solubility limits of metal ions, k_{obs} increases with increasing pH also suggests that species such as MeOH^+ is important for the metal-catalyzed hydrolysis of dimetilan since MeOH^+ concentration increases with increasing pH.

As far as **Mechanism 1** is concerned, the carbonyl oxygen has been reported to be the preferred site of metal ion coordination for both uncharged amides (44, 45) and substituted ureas (46). However, monodentate coordination of a carbonyl oxygen is generally weak, so other Lewis Base groups capable of enhancing complex formation via chelation should be sought. The nitrogen atom within the pyrazole ring (marked * in Figure 4) suits this purpose; a five-membered chelate ring is possible with the carbonyl oxygen of the substituted urea, while a six-membered chelate ring is possible with the carbonyl oxygen of the carbamate. Both rings are sterically stable, and it is possible for the two rings to form simultaneously.

Although complex formation constants involving dimetilan would be desirable, none are available. To observe trends among metal ions, another neutral ligand can be used as an analogy; a similar approach has been used previously (1, 8). The magnitudes of the complex formation constants ($\log K$) for coordinating ammonia, for example, follow the order $\text{Cu}^{\text{II}}(4.2) \gg \text{Ni}^{\text{II}}(2.9) \sim \text{Zn}^{\text{II}}(2.4) \gg \text{Pb}^{\text{II}}(1.5)$ (22). Thus, we can predict that complex formation by Cu^{II} to be strongest, Ni^{II} and Zn^{II} somewhat less but comparable, and Pb^{II} to be least. This trend is borne out in the observed trends in hydrolysis rate constants (Figure 3) if one compares either (i) the minimum pH required for measurable hydrolysis to occur or (ii) comparisons at fixed pH, as long as all four metal ions are within their solubility limits.

Metal Ion Plus EN or HEEN. Experiments investigating the effects of EN and HEEN additions on metal ion catalysis of dimetilan hydrolysis were conducted at pH 5.0 for 1.0 mM Cu^{II} and at pH 7.0 for 1.0 mM Zn^{II} . In the presence of metal ion catalysts, dimetilan loss followed pseudo-first-order kinetics, regardless of whether EN or HEEN were present.

As shown in Figure 5a, low concentrations of EN had no effect on k_{obs} for the Cu^{II} -catalyzed hydrolysis. Once concentrations surpass the total dissolved Cu^{II} concentration, however, k_{obs} diminished dramatically. In the presence of 1.0×10^{-2} M and 0.10 M EN, k_{obs} values approached the minimum quantifiable limit.

HEEN concentrations below 1.0×10^{-4} M had a similar, negligible effect on k_{obs} for Cu^{II} -catalyzed hydrolysis. HEEN concentrations between 3.0×10^{-4} M and 3.0×10^{-3} M, in contrast, resulted in k_{obs} values that were measurably higher than observed in HEEN-free solution. Higher HEEN concentrations caused k_{obs} values to drop in the same manner observed with EN. Stated differently, within an intermediate range of HEEN concentrations, Cu^{II} catalysis was enhanced.

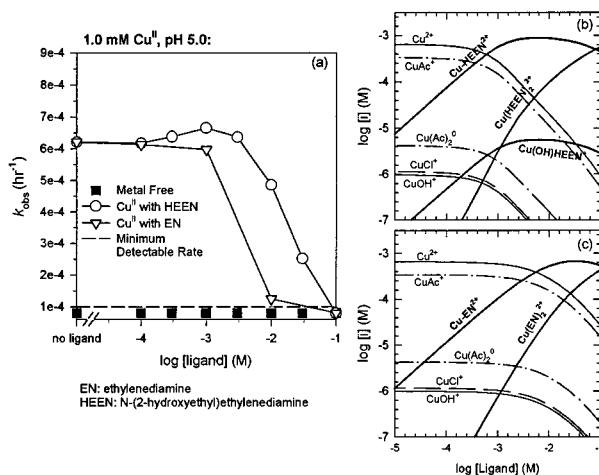


FIGURE 5. (a) Effect of EN and HEEN on Cu^{II} -catalyzed dimetilan hydrolysis at pH 5.0, 25 °C. Reaction conditions: 5.98×10^{-5} M dimetilan, 1.0 mM CuCl_2 , and 6.67 mM acetate buffer. (b) and (c) Cu^{II} speciation calculated using HYDRAQL.

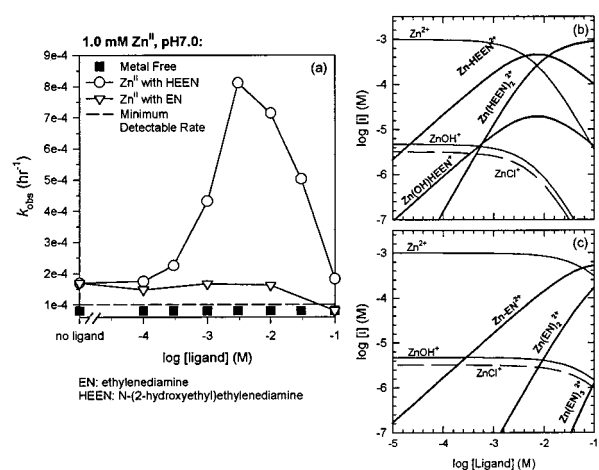


FIGURE 6. (a) Effect of EN and HEEN on Zn^{II} -catalyzed dimetilan hydrolysis at pH 7.0, 25 °C. Reaction conditions: 5.98×10^{-5} M dimetilan, 1.0 mM ZnCl_2 , and 6.67 mM MOPS buffer. (b) and (c) Zn^{II} speciation calculated using HYDRAQL.

As shown in Figure 6a, k_{obs} values in the absence of organic ligands is approximately 3.7 times lower for Zn^{II} than for Cu^{II} . The ligand EN is again able to diminish k_{obs} , although higher concentrations are needed to obtain the same effect. HEEN yields a significant increase in k_{obs} . A maximum in k_{obs} is observed at HEEN concentration near 3.0×10^{-3} M and corresponds to a nearly 4.8-fold increase in k_{obs} in comparison to HEEN-free solutions.

To assist in interpreting the results of the organic ligand addition experiments, Cu^{II} and Zn^{II} speciation has been calculated as a function of organic ligand concentration using CRITICAL (22) and HYDRAQL (39). For these experiments, metal species in solution include the species listed in eq 1 plus metal-organic ligand complexes. As a result, the k_{obs} can be expressed using the following equation

$$k_{\text{obs}} = k_{\text{Me}^{2+}}[\text{Me}^{2+}] + k_{\text{MeOH}^+}[\text{MeOH}^+] + k_{\text{MeAc}^+}[\text{MeAc}^+] + k_{\text{Me}(\text{Ac})_2^0}[\text{Me}(\text{Ac})_2^0] + k_{\text{MeCl}^+}[\text{MeCl}^+] + k_{\text{MeL}^{2+}}[\text{Me-L}^{2+}] + k_{\text{MeL}_2^{2+}}[\text{Me-L}_2^{2+}] + k_{\text{MeL}_3^{2+}}[\text{Me-L}_3^{2+}] + k_{\text{Me}(\text{OHL})^+}[\text{Me}(\text{OHL})^+] + \dots \text{etc.} \quad (2)$$

where L represents either HEEN or EN ligand. Note that experiments with Zn^{II} were conducted at pH 7.0, and thus MOPS instead of acetate buffer was used.

At the point where 1×10^{-3} M HEEN has been added, its complexes (i.e., Cu-HEEN^{2+}) dominate Cu^{II} speciation (Figure 5b). For EN, a higher concentration of 1×10^{-2} M is required to complex most of the Cu^{II} ions (Figure 5c). Although the stability constants of Cu^{II} are higher for EN than for HEEN, EN has higher basicity than HEEN (Table 1). At pHs below the pK_{a} s of the two ligands, proton competition with Cu^{II} for available ligand is more pronounced for EN than for HEEN. Thus, HEEN is a better ligand at these pHs for coordinating Cu^{II} . As indicated in Table 1, stability constants for Cu^{II} are orders-of-magnitude higher than for Zn^{II} . In order for HEEN to have the same effect on Zn^{II} , a 6.3 times higher concentration (6.31×10^{-3} M) is required (Figure 6b). Similarly, a higher concentration (0.1 M) is required for EN to complex most of the Zn^{II} ions (Figure 6c).

Comparisons between the dimetilan hydrolysis experiments and the HYDRAQL calculations indicate that 1:1 and 1:2 metal ion-EN complexes are substantially less reactive catalysts than the Cu^{II} and Zn^{II} complexes they replace (e.g., $\text{Me}^{2+}_{(\text{aq})}$, MeAc^+ , $\text{Me}(\text{Ac})_2^0$, etc.). With HEEN, the situation is quite different. The 1:1 metal ion-HEEN are apparently more reactive catalysts than the Cu^{II} and Zn^{II} complexes they replace, while 1:2 complexes are substantially less reactive. Stating this differently, the pronounced enhancement effect arising from HEEN addition can be attributed to the 1:1 complex, while the 1:2 complex inhibits metal ion-catalyzed hydrolysis.

Since EN and HEEN have very similar $\log K$ values for metal ion complexation, differences in their effect on metal ion-catalyzed hydrolysis must arise from the hydroxyl group. The fact that HEEN does not influence dimetilan hydrolysis in the absence of metal ions indicates that HEEN can only exert a catalytic effect on dimetilan hydrolysis cooperatively with metal ions (i.e., a synergistic effect of metal ion and HEEN). Coordination of the additional hydroxyl group of HEEN to the central metal ion is possible since the hydroxyl group is suitably placed for five-membered chelate ring formation to occur.

There are three possibilities which can explain the effect of HEEN: (A) metal ion complexation activates HEEN, making it a reactive nucleophile or a general base catalyst; (B) metal ion complexation activates dimetilan, making it susceptible toward nucleophilic attack by HEEN; or (C) the metal ion complexes both HEEN and dimetilan; in the ternary complex, the hydroxyl group of HEEN facilitates dimetilan hydrolysis as an intramolecular nucleophile or general base catalyst.

Of the above possibilities, (B) is least likely based upon two reasons. First, the pK_{a} of the HEEN hydroxyl group is so high that it is an improbable nucleophile or general base catalyst under the experimental conditions used. Second, HEEN is very likely to be complexed with Cu^{II} and Zn^{II} , since the $\log K$ values for both metal ions are quite large and enhancement in catalysis is observed even when the HEEN concentration is less than the metal ion concentration.

Option (C) is considered to be more likely than (A), since the experimental results with the four divalent metal ions strongly indicate that coordination of dimetilan with metal ions is crucial for metal ion catalysis to occur. Furthermore, the combined ("push-pull") mechanism in which the metal ion simultaneously coordinates the substrate and the nucleophile occurs frequently (40–43). Therefore, (C) is the most plausible explanation.

Compared to HEEN, EN lacks the hydroxyl functional group that can act as an auxiliary nucleophile within the metal ion-EN-dimetilan ternary complex. Therefore, EN addition decreases metal catalysis. For both EN and HEEN, the 1:2 complexes of metal and ligands are substantially less reactive catalysts because excess ligands occupy most coordination sites of metal ions, preventing the formation of metal ion-organic ligand-dimetilan ternary complexes.

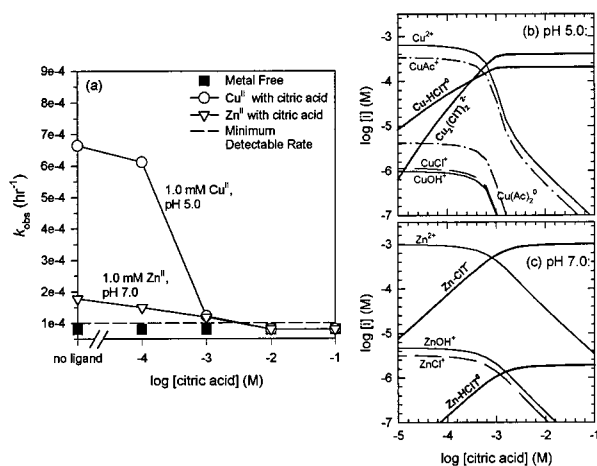


FIGURE 7. (a) Effect of citric acid ($\text{CIT} = \text{L}^{3-}$) on Cu^{II} - and Zn^{II} -catalyzed dimetilan hydrolysis. Reaction conditions: 6.96×10^{-5} M dimetilan, 1.0 mM CuCl_2 or ZnCl_2 , 0.02 M acetate (pH 5.0) or MOPS (pH 7.0) buffer, 25 °C. (b) and (c) Cu^{II} and Zn^{II} speciation calculated using HYDRAQL.

It is interesting that HEEN exerts a much greater enhancement effect on Zn^{II} catalysis than Cu^{II} catalysis. However, this difference could arise from the different pHs that the experiments were conducted or from the property differences between Cu^{II} and Zn^{II} . The experiments with Cu^{II} were not conducted at pH 7 due to concern of the solubility limit of Cu^{II} . To fully understand differences between Cu^{II} -HEEN systems and Zn^{II} -HEEN systems, experiments such as the ones shown in Figures 5 and 6 should be conducted at several pHs and using several dissolved metal ions concentrations.

Metal Ion Plus CIT. The effect of CIT additions on metal ion catalysis of dimetilan hydrolysis was examined at pH 5.0 for 1.0 mM Cu^{II} and at pH 7.0 for 1.0 mM Zn^{II} . In the presence of metal ion catalysts, dimetilan loss followed pseudo-first-order kinetics, regardless of whether CIT was present.

As shown in Figure 7a, 1.0×10^{-4} M CIT slightly decreased k_{obs} for both the Cu^{II} - and Zn^{II} -catalyzed hydrolysis. When CIT concentrations reached 1.0×10^{-3} M and above, k_{obs} values diminished dramatically. In the presence of 1.0×10^{-2} M and 0.10 M CIT, k_{obs} values approached the minimum quantifiable limit.

Cu^{II} and Zn^{II} speciation has been calculated as a function of CIT concentration using CRITICAL (22) and HYDRAQL (39). At the point where 1×10^{-3} M CIT has been added, CIT complexes dominate Cu^{II} speciation (Figure 7b). At a concentration slightly higher than 1×10^{-3} M, CIT complexes dominate Zn^{II} speciation (Figure 7c).

Comparisons between the dimetilan hydrolysis experiments and the HYDRAQL calculations indicate that 1:1 and 1:2 metal ion-CIT complexes are substantially less reactive toward dimetilan than the Cu^{II} and Zn^{II} complexes they replace (e.g., $\text{Me}^{2+}_{(\text{aq})}$, MeAc^+ , $\text{Me}(\text{Ac})_2^0$, etc.). The carboxylate groups of CIT allow it to occupy three coordination sites of metal ions, which may effectively prevent ternary complex formation. Alternatively, it is possible that ternary complexes form but possess no unusual reactivity; CIT coordination may interfere with the ability of the metal ion to activate dimetilan toward hydrolytic attack (**Mechanism 1**) or may interfere with the ability of the metal ion to generate a reactive nucleophile through the induced deprotonation of water (**Mechanism 2**). Although induced deprotonation of the hydroxyl group of CIT was observed with Fe^{III} (15), Cu^{II} and Zn^{II} , which are less Lewis acidic than the trivalent metal ions, are not believed to have the same ability. Thus, generation of a reactive intramolecular nucleophile, possible with HEEN, is not possible with CIT.

To the author's knowledge, this is the first study of metal ion-organic ligand synergistic effects on the hydrolysis of agrochemicals. Out of a long list of possible synergistic effects, our experiments provide examples of only a few. EN and CIT, both capable of coordinating +II metal ions but lacking possible intramolecular nucleophilic groups, inhibit metal ion-catalyzed dimetilan hydrolysis. Simple carboxylate ligands similar to CIT (e.g., oxalic acid) are expected to lower the catalytic effects of metal ions toward dimetilan hydrolysis. It is possible, however, that other organic ligands exist which lack intramolecular nucleophilic groups but nevertheless facilitate catalysis (i.e., by encouraging ternary complex formation with substrate or by facilitating shifts in electron density that activate the substrate toward hydrolysis).

HEEN, capable of coordinating +II metal ions and possessing a hydroxyl group that can serve as an intramolecular nucleophile, significantly facilitates metal ion-catalyzed dimetilan hydrolysis. Moderately- and highly basic functional groups (e.g., amines, phenols, thiols and hydroxyls) near metal ion-binding moieties are found in many natural products and can be postulated to occur within humic substances. As mentioned earlier, extracellular enzymes are already known to take advantage of metal ion-organic ligand synergistic effects. It is quite possible that partially degraded enzymes and other compounds released by cell lysis would exhibit similar properties. As we learned more about the identity and concentrations of metal ions and ligands in pertinent microenvironments (e.g., the rhizosphere surrounding roots and other soil interstitial solutions), we will be in a better position to evaluate synergistic or inhibitory effects on the degradation of agrochemicals. Hence, future studies of metal ion-organic ligand synergistic effects on the hydrolysis of agrochemicals are likely to be fruitful.

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