PROELECTROPHILE-NUCLEOPHILE ADDUCT FORMATION AND PERMANGANATE OXIDATION OF ALKENES: USE OF MODEL COMPOUNDS TO PROBE COMPLEX REACTION PATHWAYS

By

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Abstract

Laboratory studies of reactions between model compounds yield valuable insights into complex reaction chemistries that would be difficult if not impossible to observe directly in environmental media. We used model compounds to investigate two types of reaction pathways: adduct formation and permanganate oxidation.

First, we mimicked covalent bond forming reactions between nucleophilic contaminants and electrophilic sites within natural organic matter (NOM) or naturally occurring electrophilic toxicants. Initial studies modeled adduct formation at electrophilic NOM sites by reacting the model electrophiles p-benzoquinone and patulin with a suite of model nucleophiles. Further studies used MnO$_2$(s, pyrolusite)-oxidized hydroquinone proelectrophiles to simulate the highly electrophilic moieties that can be generated when NOM reacts with oxidants in a sediment or soil. Overall, our model compound studies demonstrated that added nucleophiles compete with H$_2$O/OH$^-$ to form monoadducts, which can then undergo further oxidization and addition to form di- and triadducts. Mass spectra consistent with monoadducts from reactions between nine nucleophiles and MnO$_2$-oxidized gentisic acid were acquired by LC/MS. Separate experiments monitoring both the consumption of a model proelectrophile and a nucleophile (acetylhydroquinone and 4-ethylaniline, respectively) and the generation of Mn$^{II}$ from reduction of MnO$_2$ provided indirect evidence for multiple oxidation-addition steps.
Second, we used model compounds to study permanganate oxidation of alkenes. Competition between hydrolysis and oxidation of a common intermediate leads to a distribution of products. To collect direct evidence of the influence of pH and oxidant dose on this product distribution, we selected cis-stilbenedicarboxylic acid as a model compound because it yields permanganate oxidation products amenable to LC/MS analysis. High permanganate dose favors highly oxidized products, while alkaline pH favors products from hydrolysis.

This dissertation demonstrates how strategic selection of model compounds can elucidate complex environmental contaminant removal pathways by providing an internally consistent system for the study of multiple reaction conditions and competitive reactions.

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Readers: A. Lynn Roberts and Steven Rokita
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List of Symbols

AAS       Flame Atomic Absorption Spectrometry
An        4-Ethylaniline
CHES      N-Cyclohexyl-2-aminoethanesulfonic acid
DEPP      1,4-Diethylpiperazine
e−        Electron
EDG       Electron Donating Group
EDTA      Ethylenediaminetetraacetic acid
EWG       Electron Withdrawing Group
HPLC      High Performance Liquid Chromatography
[i]       Concentration of i, in either mol/L or µMol/L
[i]₀      Initial concentration of i, in either mol/L or µMol/L
Im        4-Methylimidazole
k_{obs}   Observed Pseudo-First-Order Rate Constant
LC/MS     Liquid Chromatography with Mass Spectrometry
LOD       Limit of Detection
m/z       Mass to Charge ratio of a fragment detected in mass spectrometry
MES 2-(N-morpholino)ethanesulfonic acid
MOPS 3-(N-morpholino)propanesulfonic acid

\([\text{M-COOH}]^-\) Anionic fragment produced during ionization in LC/MS resulting from loss of a carboxylic acid functional group

\([\text{M-H}]^-\) Anionic fragment produced during ionization in LC/MS resulting from loss of a carboxylic acid functional group

NER Non-Extractable Residue
NMR Nuclear Magnetic Resonance
NOM Natural Organic Matter
Nu Nucleophile
OE Oxidizing Equivalent

\(pK_a\) Acid Dissociation Constant

UV/Vis Ultraviolet/Visible Light Spectrophotometry

X-Nu Monoadduct hydroquinone resulting from addition of an added nucleophile

X-OH Monoadduct hydroquinone resulting from addition of \(H_2O\) or \(OH^-\)

\(XH_2\) A \(p\)-dihydroxybenzene

\(XH_2(I)\) Hydroquinone

\(XH_2(II)\) Acetylhydroquinone

\(XH_2(III)\) 2,5-Dihydroxybenzoic acid

\(XH_2(IV)\) 2,5-Dihydroxyterephthalic acid

\(Z\) A \(p\)-benzoquinone

\(Z-Nu\) Monoadduct benzoquinone resulting from oxidation of X-Nu
<table>
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Chapter 1

Introduction

1.1 Motivation

Manganese can be an important oxidant in soils and sediments and in water and wastewater treatment systems. We will focus on Mn$^{III,IV}$ (hydr)oxides and permanganate (MnO$_4^-$). Mn$^{III,IV}$ (hydr)oxides are present in both sediments and soil and have high enough reduction potentials to oxidize many organic reductants such as hydroquinone and catechols, which could represent redox active moieties within natural organic matter (NOM).

Contaminants containing nucleophilic functional groups could be removed from the water column by undergoing nucleophilic addition reactions with electrophilic sites within NOM. Whether or not removal is irreversible depends on the reaction mechanism and the nature of that product that is ultimately generated. Most organic contaminants will partition into NOM based on their affinity for organic matter versus aqueous solution, however this “physical” partitioning phenomenon is reversible and can be described by partitioning coefficients that are often correlated to the octanol-water partition coefficient ($K_{ow}$). Mechanisms that lead to covalent bonds and thus irreversible sorption include radical coupling, nucleophilic substitution, and nucleophilic addition reactions. Nucleophilic
addition can form a bond between nucleophiles and carbonyl functional groups (ketones and aldehydes) or \(\alpha,\beta\)-unsaturated carbonyls, also known as "Michael acceptors." Whether or not a contaminant will form an irreversible bond depends on the strength of the nucleophile and the ambient chemical conditions.

The importance of nucleophilic addition reactions in contaminant removal also depends on the strength of the electrophilic functional groups. NOM is thought to contain carbonyl and benzoquinone electrophilic functional groups (1, 2). Nucleophilic addition to carbonyl functional groups, 1,2-addition, is reversible but Michael-type addition, 1,4-addition, to benzoquinones is irreversible (3, 4). Both 1,2- and 1,4-addition will occur with NOM, but 1,4-addition to benzoquinone electrophiles leads to ultimate removal of the contaminant (3).

The electrophilicity of a benzoquinone functional group changes based on whether it is a 1,4 or a 1,2 benzoquinone and on electron withdrawing and donating ability of the ring substituents (5). The NOM matrix to which a benzoquinone is attached will exert both electronic and steric effects on its electrophilicity. Benzoquinones deep within the NOM structure may be unavailable for reaction with nucleophiles from the water column for steric reasons. Benzoquinones bound to NOM via alkyl chains are inhibited by the electron donating properties of alkyl functional groups. However, carbonyl groups such as carboxylic acids are electron withdrawing and should increase the electrophilicity of nearby benzoquinones. Ratasuk et al. (2) suggested that quinone functional groups within NOM fall into two categories: reactive benzoquinones that possess electron-withdrawing groups, and sterically hindered benzoquinones that are bonded to electron-donating substituents (2).

Benzoquinone functional groups within NOM are also thought to be involved in the redox properties of NOM (2, 6–9). For example, some microorganisms use NOM as an intermediate electron shuttle, allowing them to reduce insoluble Fe(III) to bioavailable Fe(II) (9, 10). Benzoquinones are linked to catechol and hydroquinone moieties by reduction
and oxidation reactions: oxidation of NOM will transform catechol and hydroquinone moieties into benzoquinone, reduction of NOM transforms benzoquinones into phenols. This means that the presence of an oxidant will generate electrophilic benzoquinone moieties via oxidation of non-electrophilic catechol and hydroquinone moieties. We refer to catechol and hydroquinone moieties as “proelectrophiles” which are compounds that become electrophilic after oxidation. We therefore expect that oxidation of NOM will increase the number of electrophilic sites within NOM.

Beyond the number of electrophilic sites, we expect that oxidation will also increase the electrophilicity of NOM. Benzoquinones with electron-withdrawing substituents are highly unstable in the presence of water. For example, carboxy-p-benzoquinone cannot be synthesized (11) and is not commercially available. Carboxy-p-benzoquinone is so electrophilic that it quickly reacts with an available nucleophile. In aqueous solution, hydroxide ion and water itself can both react as nucleophiles. We refer to addition of water or hydroxide as “hydration.” When a stronger nucleophile is available, addition products other than the hydration product will be formed. Oxidation could unlock highly reactive electrophilic sites within NOM by generating benzoquinone sites with electron-withdrawing substituents that can react with nucleophilic contaminants.

Permanganate (MnO$_4^-$) is used in many ways in water treatment and environmental remediation (12, 13). MnO$_4^-$ can remove Mn$^{II}$ and Fe$^{II}$ during drinking water treatment as well as remedy taste and odor problems. It can remove precursors to trihalomethanes and other disinfection byproducts. It can promote coagulation and flocculation improving removal of organic carbon. MnO$_4^-$ can also be used as a chemical oxidant in groundwater remediation (14).

Although manganese is an essential nutrient and is found naturally in source waters, too much manganese in drinking water can cause problems with unpleasant taste or odor,
discoloration, and staining of plumbing fixtures, leading to customer complaints for drinking water suppliers (15, 16). Excess iron leads to similar complaints. Water containing high concentrations of manganese usually comes from wells under reducing conditions where manganese and iron exist in their reduced and soluble forms: Mn$^{2+}$ and Fe$^{2+}$ (17).

Drinking water treatment plants remove excess manganese and iron by one of two methods. Adding an oxidant (such as MnO$_4^-$, ozone, or chlorine dioxide) will oxidize Mn$^{2+}$ and Fe$^{2+}$ to insoluble forms. The resulting particles can be removed by filtration or coagulation and sedimentation. A second method called “manganese green sands” involves a column filter loaded with Mn$^{III,IV}$ (hydr)oxide coated media. The Mn$^{III,IV}$ coating oxidizes Mn$^{2+}$ and Fe$^{2+}$ to insoluble forms that the filter media traps. Continuous or pulse addition of an oxidant (usually MnO$_4^-$ or free chlorine) regenerates Mn$^{IV}$ in the oxide coating (17–21).

Similarly, MnO$_4^-$ can be used to help remove arsenic from drinking water. MnO$_4^-$ oxidizes arsenite (As$^{III}$) to arsenate (As$^{V}$). Precipitation or adsorption can remove arsenate but arsenite is difficult to remove using these conventional techniques (22). Arsenic removal from drinking water is a critical public health issue. Chronic exposure to arsenic in drinking water at high concentrations can lead to skin or lung cancer, while at low concentrations it can cause chronic fatigue, hair loss, weight loss, variation in skin pigment, hyperkeratoses, and ulcerations (22, 23).

Some water treatment plants use MnO$_4^-$ to remove taste and odor problems from drinking water (15, 16). Organic odor compounds of algal or bacterial origin such as β-cyclocitral (24, 25) and dimethyl trisulfide (26) are degraded by MnO$_4^-$. MnO$_4^-$ effectively treats source waters contaminated with hydrogen sulfide (27). MnO$_4^-$ can also be used in industrial wastewater treatment. For example, it effectively decolorizes dye and textile industry wastewater (28).
Oxidation of organic matter in source waters can remove precursors that form trihalomethanes and other disinfection byproducts (29–31). Many of these byproducts, including trihalomethanes, are suspected carcinogens and are regulated by the Clean Water Act. In drinking water treatment, THMs are generated by oxidation of organic matter by free chlorine. As concern about disinfection byproducts in drinking water increases and regulatory limits tighten, permanganate pre-oxidation may be used in more drinking water treatment plants to control DBP formation.

Some “enhanced coagulation” techniques employ MnO$_4^-$ oxidation, which improves aggregation during coagulation in water treatment plants, especially when the source water contains high levels of organic carbon. MnO$_4^-$ aids aggregation in two ways: it breaks up organic matter into smaller fragments that are more easily adsorbed by coagulant (32), and it precipitates solid Mn$^{III,IV}$ oxyhydroxide particles that aid in adsorbing organic matter or that cause further oxidation (33, 34).

“In-situ chemical oxidation” employs MnO$_4^-$ or another oxidant for groundwater remediation. Oxidant solution is delivered by a pump and flood method where MnO$_4^-$ reacts with the target contaminant (35). In-situ chemical oxidation using MnO$_4^-$ as an oxidant can effectively treat many contaminants including chlorinated ethylenes (36–38), cyclotrimethylenetrinitramine (RDX, 39, 40), chlorophenols (41), and methyl-tert-butyl ether (42). Waldemer and Tratnyek (43) reported rate constants for 24 contaminants including some pesticides, substituted phenols, TNT, and methyl ethyl ketone.

Researchers are exploring the ability of MnO$_4^-$ to remove micropollutants from water. Some micropollutants that are effectively removed by MnO$_4^-$ include the cyanobacterial toxin microcystin-LR (44–47), endocrine-disrupting compounds related to estradiol (48), the antibacterial compound triclosan (49), and pesticides including aldrin, terbufos, metribuzin, permethrin (50), and dichlorvos (51).
1.2 Oxidation States of Manganese

The manganese atom in MnO$_4^-$ is in the +7 oxidation state which is the highest oxidation state of manganese (Figure 1.1). The standard reduction potential of permanganate is based on which reduction product is generated which is thought to depend on solution pH (Table 1.1).

The +3 and +4 Mn oxidation states exist as solids in relevant aqueous conditions forming a variety of Mn$^{III,IV}$ (hydr)oxides that vary by crystal structure and Mn$^{III}$ to Mn$^{IV}$ ratio (52, 53). These compounds are important in environmental reduction and oxidation reactions and are nearly ubiquitous in soils and sediments (53). Mn$^{II}$ exists as a colorless and highly soluble ion, Mn$^{2+}$(aq).

Mn$^V$ and Mn$^VI$ could be important species involved in reduction of MnO$_4^-$, but only transiently because they are generally not stable under conditions relevant to water and wastewater treatment. Manganate (Mn$^{VI}$) exists as an oxoanion, MnO$_4^{2-}$, that is unstable in water and disproportionates to MnO$_2$ and MnO$_4^-$ (54).

$$3\text{Mn}^{VI}O_4^{2-} + 2\text{H}_2\text{O} \rightleftharpoons \text{Mn}^{IV}O_2 + 2\text{Mn}^{VII}O_4^- + 4\text{OH}^- \quad (1.1)$$

The equilibrium constant for Equation 1.1 favors MnO$_2$ and MnO$_4^-$. MnO$_2$ particles act as a heterogeneous catalyst and increase the rate of disproportionation (55). Hypomanganate (Mn$^V$) is stable as MnO$_4^{3-}$ in highly alkaline solution, although the protonated species rapidly reacts via disproportionation which is catalyzed by MnO$_2$ solid (56). Additionally, one-electron oxidation of hypomanganate to manganate is “immeasurably fast at room temperature” (56).

$$\text{MnO}_4^- + \text{MnO}_4^{3-} \rightleftharpoons 2\text{MnO}_4^{2-} \quad (1.2)$$
Mn$^{\text{III}}$ and Mn$^{\text{IV}}$ precipitate as (hydr)oxide solids in aquatic conditions. Pyrolusite ($\beta$-MnO$_2$) is the most thermodynamically stable form under oxidizing conditions at circumneutral to alkaline pH (52). Pyrolusite ($\beta$-MnO$_2$), feitnechtite ($\beta$-MnOOH), manganite ($\gamma$-MnOOH), and birnessite ($\delta$-MnO$_2$) all have high reduction potentials, making them powerful oxidants. Reduction potentials for some Mn$^{\text{III,IV}}$ (hydr)oxide are shown in Table 1.1.

In aquatic environments manganese cycles across the oxic/anoxic interface because of the differing properties of the +3/+4 and +2 oxidation states (Figure 1.2, 57). Mn$^{\text{III,IV}}$ (hydr)oxides are stable in the oxic zone of the water column and settle to the top of sediments. Abiotic and microbial reactions reduce the (hydr)oxides to Mn$^{2+}$(aq) which is is highly soluble in water and readily diffuses back into the water column. Fluxes of Mn$^{2+}$(aq) from sediments have been measured in several different locations and range from 0.07 to 2.3 mmol m$^{-2}$ day$^{-1}$ (58). After returning to the oxic zone, Mn$^{2+}$(aq) is oxidized via both microbial and abiotic mechanisms into Mn$^{\text{III,IV}}$(hydr)oxide particles (58, 59) to complete the cycle. Due to this redox cycling of manganese, each manganese atom is oxidized and reduced 100 to 300 times before ultimate burial (60).

Mn$^{\text{III,IV}}$(hydr)oxides can be reduced microbially (61, 62), by sulfide below the oxic/anoxic interface (63), or by organic molecules (64–66). Reduction by dissolved Fe$^{\text{II}}$ is also possible, but is inhibited by complexation of Fe$^{2+}$ (62).

Oxidation of Mn$^{2+}$(aq) is favorable under oxidizing conditions at circumneutral pH (52). Oxidation by O$_2$ occurs slowly in solution or at a mineral surface (67). Microbial oxidation is several orders of magnitude faster, and increases with increasing concentration of dissolved O$_2$ (68–71).
1.3 Research Objectives and Thesis Outline

This research is focused on three reaction pathways: Michael addition of nucleophiles to benzoquinones and naturally occurring Michael acceptors, the proelectrophile reaction mechanism, and permanganate oxidation of alkenes. We are particularly interested in the effect of aqueous chemical conditions (especially pH) and initial concentrations on the resulting product distribution. We chose to work primarily with model compounds in clean laboratory conditions. Using model compounds allows us to directly study kinetics and mechanisms involved in all three pathways which is prohibitively difficult when using environmental samples. Also, working with model compounds decreases the complexity of the product distribution generated by these reactions.

In Chapter 2, we employ \( \textit{p} \)-benzoquinone and patulin as model Michael acceptor electrophiles to improve our understanding of the kinetics and mechanism of the Michael addition reaction in neutral pH aqueous conditions. The rate of electrophile consumption under pseudo-first-order kinetic conditions gives a direct comparison of the nucleophilicity towards Michael addition of our model nucleophiles. We show that the Hammett relationship holds for para-substituted anilines for Michael addition, indicating the important of electronic effects on reaction rate.

Chapter 3 explores the proelectrophile pathway, which we expect will occur in sediments (1, 3, 4, 72) and soils (73, 74). Our model electrophiles are hydroquinones containing electron-withdrawing group substituents. The hydroquinone molecules are oxidized by \( \text{MnO}_2(s, \text{pyrolusite}) \) particles that are 98% \( \text{Mn}^{IV} \) and only 2% \( \text{Mn}^{III} \), so we expect a one-to-one stoichiometry between hydroquinone and \( \text{MnO}_2 \). Oxidation generates a highly electrophilic benzoquinone that quickly reacts with water to form a hydration product or an
added nucleophile to form an adduct. We prepared solutions containing nine nucleophiles and gentisic acid in the presence of MnO₂ and recorded LC/MS evidence for the resulting addition products. Because benzoquinone adducts and hydration products are themselves hydroquinones, they are subject to oxidation by MnO₂, yielding a second generation of benzoquinones that can undergo further hydration and nucleophilic reaction steps. It is, therefore, possible for one hydroquinone model compound to undergo oxidation and addition multiple times in the presence of excess oxidant. We show that the importance of second generation addition products depends on the initial concentration of nucleophile.

In Chapter 4, we employ cis-stilbenedicarboxylic acid as a model compound to understand product distribution resulting from alkene oxidation by MnO₄⁻. The oxidation rate of alkene functional groups by MnO₄⁻ is fast and independent of pH for the rate-determining step which is thought to be attack of MnO₄⁻ attacks on the alkene resulting in an unstable intermediate. The product distribution results from a complex system of reactions that break down the intermediate. Several pathways are possible and which of the pathways dominates depends on competition between hydrolysis and further oxidation steps. The possible organic oxidation and Mn reduction products differ by the extent of oxidation that occurs. The organic oxidation products range from diols to a pair of carboxylic acids from C=𝐶 bond-cleavage. We use LC/MS to monitor organic reactants and products including products for which authentic standards are not available. We propose a modified iodometric titration method to measure MnO₄⁻, Mn^{III,IV} solids, and Mn²⁺. We vary the pH, the buffer system, and reductant to oxidation concentration ratio to explore their effects on the product ratios.
References


(33) J. Ma, G. Li. Laboratory and full-scale plant studies of permanganate oxidation as an aid in coagulation. *Water Science and Technology* 1993, 27, 47–54.


Table 1.1. Reduction half-reactions and standard reduction potentials for environmentally and water/wastewater treatment relevant manganese species (37, 52, 56, 75, 76). For MnO$_4^-$ the relevant pH range is given (37, 56).

<table>
<thead>
<tr>
<th>Reduction Half-Reaction</th>
<th>$E^\circ$ (V)</th>
<th>relevant pH range</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnO$_4^-$ + e$^-$ $\rightleftharpoons$ MnO$_2^{2-}$</td>
<td>+0.56</td>
<td>$&gt;$ 12</td>
</tr>
<tr>
<td>MnO$_4^-$ + 3 e$^-$ + 2 H$_2$O $\rightleftharpoons$ MnO$_2$ + 4 OH$^-$</td>
<td>+0.59</td>
<td>7–12</td>
</tr>
<tr>
<td>MnO$_4^-$ + 3 e$^-$ + 4 H$^+$ $\rightleftharpoons$ MnO$_2$ + 2 H$_2$O</td>
<td>+1.70</td>
<td>3.5–7</td>
</tr>
<tr>
<td>MnO$_4^-$ + 5 e$^-$ + 8 H$^+$ $\rightleftharpoons$ Mn$^{2+}$ + 4 H$_2$O</td>
<td>+1.51</td>
<td>$&lt;$ 3.5</td>
</tr>
<tr>
<td>$\beta$–MnOOH(s) + 3 H$^+$ + e$^-$ $\rightleftharpoons$ Mn$^{2+}$(aq) + 2 H$_2$O</td>
<td>+1.65</td>
<td></td>
</tr>
<tr>
<td>$\gamma$–MnOOH(s) + 3 H$^+$ + e$^-$ $\rightleftharpoons$ Mn$^{2+}$(aq) + 2 H$_2$O</td>
<td>+1.50</td>
<td></td>
</tr>
<tr>
<td>$\frac{1}{2} \delta$–MnO$_2$(s) + 2 H$^+$ + e$^-$ $\rightleftharpoons$ $\frac{1}{2}$ Mn$^{2+}$(aq) + H$_2$O</td>
<td>+1.29</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1.1. Oxidation states of manganese relevant to environmental and water/wastewater treatment conditions.
Figure 1.2. Manganese distribution (Left) and cycle around the oxic/anoxic interface (right, 57). 1) Gravitational settling causes Mn_{III,IV} (hydr)oxide particles to sink below the interface. 2) There, they are reduced by microorganisms or abiotically to Mn^{2+}(aq). 3) Highly soluble Mn^{2+}(aq) readily diffuses into the oxic zone. 4) Once in the oxic zone, Mn^{2+}(aq) can be oxidized back to Mn_{III,IV} solids by microorganisms or O_2.
Chapter 2

Aqueous Addition Reactions of Patulin and

\[ p \]-Benzoquinone: Competition Between Added

Nucleophiles, Water, and Buffers

2.1 Introduction

Organic contaminant interactions with natural organic matter (NOM) can occur via several different mechanisms including ionic, hydrogen and covalent bonding, charge-transfer or electron donor-acceptor mechanisms, van der Waal forces, ligand exchange, hydrophobic effects, or partitioning (1). Contaminants that cannot be recovered from a soil by extraction are called bound residues or nonextractable residues (NER). Relative to the parent contaminant, NER have lower bioavailability and mobility and their formation could represent a safe and effective removal mechanism for some contaminants (1–3).

Contaminants containing nucleophilic functional groups like aniline, phenol, and N-heterocycles could form NER via covalent bonding, and the extent of NER formation is sensitive to molecular structure of the contaminant (1, 4). Based on kinetic data for pesticides, those containing phenol or aniline nucleophilic functional groups exhibit greater
NER formation than those containing N-heterocycles (4). In one study where $^{14}$C-labeled pyridine and aniline were incubated with a sediment sample, pyridine was almost completely recovered by multiple extractions but aniline could only be partially recovered (5). Aniline loss is thought to occur via both reversible mass-transfer sorption interactions, cation exchange reactions, and covalent bond forming reactions representing NER formation (5, 6). Aniline contains an amine functional group and could form covalent bonds via a nucleophilic addition reaction, while pyridine is a poorer nucleophile (5). NMR and mass spectrometric studies confirmed the formation of covalent bonds between NOM and aniline (7).

Michael addition is one pathway that could lead to irreversible covalent bond formation between nucleophilic contaminants and NOM (8–11). The Michael addition reaction occurs between a nucleophile and a “Michael acceptor” electrophile containing an $\alpha, \beta$-unsaturated carbonyl substructure (–C=–C=O). This reaction can also be called 1,4-addition because the nucleophilic attack occurs at the $\beta$ carbon, but the donated electrons are transferred to the oxygen atom that is four atoms away.

$$\text{Nu} \rightarrow \begin{array}{c} \text{Nu} \\
\text{2} \\
\text{3} \\
\text{4} \\
\text{O} \\
\end{array} \rightarrow \begin{array}{c} \text{Nu} \\
\text{2} \\
\text{3} \\
\text{4} \\
\text{O} \\
\end{array}$$

where Nu is the nucleophile. Figure 2.1 depicts Michael addition to p-benzoquinone (8). The reaction is initiated by nucleophilic attack at the $\beta$-carbon. Proton transfer and keto-enol tautomerization reactions lead to the final products. The molecular structure of both the nucleophile and the Michael acceptor affect rates of Michael addition. The strength of a nucleophile is related to the electron density at the atom that donates electrons during the attack step. This atom is called the nucleophilic center. Structure effects that increase electron density at the electrophilic atom should increase the rate of Michael addition.
reactions. The reverse is true for Michael acceptors, the carbonyl withdraws electron-density at the receptor site making them electron deficient.

NOM is thought to contain $\alpha$- and $\beta$-benzoquinone moieties that could be active participants in many biogeochemical cycles (12, 13). Similarities between NOM samples and model quinones have been reported as evidence of benzoquinone moieties using cyclic voltammetry (14), redox titration (15), cyclic $O_2$ oxidation and $H_2$Pd reduction experiments (16), and fluorescence spectroscopy (17).

A nucleophile could form an irreversible bonds to an $\alpha,\beta$-unsaturated carbonyl in a benzoquinone moiety within NOM via Michael addition. $^{15}N$ NMR and kinetic studies provide evidence for bond formation by aniline nucleophiles (7, 8). Direct evidence of benzoquinone moieties can be obtained by $^{15}N$ and $^{13}C$ NMR spectroscopy of fulvic and humic acid samples that have reacted with $^{15}N$-labeled hydroxylamine (18). Although the less stable ortho-benzoquinone isomer is more likely to be present in NOM, we will employ $p$-benzoquinone as a convenient model electrophile for laboratory experiments (19). $p$-Benzoquinone could be present in receiving waters of disinfected wastewater as a result of chlorination of municipal wastewater containing acetaminophen (20).

The mycotoxin patulin (Figure 2.2) is used as a second model Michael acceptor electrophile. Patulin is a naturally-occurring electrophilic toxic compound arising from certain *Penicillium* and *Aspergillus* fungal species that grow on rotting crops, especially apples and pears (21–24). Mycotoxins can leach into surface water from from decaying agricultural fields (25, 26). Patulin is carcinogenic and teratogenic (27, 28) and can induce intestinal injuries such as degeneration of epithelial cells, inflammation, ulceration, and hemorrhages (27). Nucleophilic addition reactions of patulin with biological thiol nucleophiles such as cysteine and glutathione provide protection from toxicity of patulin and generate less toxic adducts (27–29).
Our study employs model nucleophiles, para-substituted anilines, 4-methoxyphenol, hydroxylamine, and 4-methylimidazole, to study nucleophilic addition reactions. These model compounds serve to represent aromatic amine, phenolic, and heterocyclic nitrogen functional groups that can impart nucleophilicity to organic contaminants. 4-Methylimidazole serves as a model for histidine and similar heterocyclic nitrogen compounds. These model nucleophiles are experimentally convenient because they contain only one Lewis base group that could donate lone-pair electrons during nucleophilic attack. More complicated compounds can contain two or more nucleophilic sites. For example, the herbicides clopyralid, chloramben and aminopyralid contain potentially nucleophilic pyridine-like and aniline-like functional groups and may form NERs via Michael addition. Clopyralid and aminopyralid are used to control broadleaf weeds (30, 31). Clopyralid (30) and aminopyralid (31) do not leach from test agricultural plots, do not hydrolyze, and do not significantly volatilize, but they cannot be detected in the soil within months of application. In these field studies the loss of clopyralid and aminopyralid was credited to biodegradation. However, an abiotic mechanism involving covalent bond formation with soil organic matter could have evaded the detection method. In order to determine if herbicides like clopyralid and aminopyralid are lost by nucleophilic addition reactions, it would be useful to know how nucleophilic these compounds are. We propose that rate of loss of p-benzoquinone measured in a simple laboratory experiment could be used as a tool to assess nucleophilicity towards addition by complicated nucleophiles that may correlate with reaction rate with NOM.

We apply the Hammett relationship to describe the effect of electronic structure on rate constant \( (k_{Nu^-}) \) for loss of p-benzoquinone in the presence of para-substituted anilines. These nucleophiles have only one Lewis base group allowing a Hammett relationship to be developed to describe substituent effects on rate of loss of electrophile. The Hammett relationship (32, 33) can be applied to many thermodynamic or kinetic constants by relating...
the log of a rate or equilibrium constant to a reaction constant ($\rho$) and substituent constants ($\sigma$). We use it for second-order rate constants of Michael acceptor loss attributed to the nucleophiles deprotonated form $k_{\text{Nu}^-}$:

$$\log(k_{\text{Nu}^-}) = \rho \sigma$$  \hspace{1cm} (2.2)

The reaction constant is determined from the slope of Equation 2.2 and is used as a way to quantify electronic structure sensitivity of Michael addition reactions with $p$-benzoquinone and patulin model Michael acceptors and para-substituted aniline nucleophiles. Some Hammett $\rho$ values are available in the literature for reaction of para-substituted anilines with soil samples (8, 11, 34) and with model electrophiles (8, 35). Colón and coworkers (11) observed biphasic kinetics characterized by a fast first step and a second slow step for loss of a suite of aromatic amines added to sediment samples. They found good correlation between Hammett substituent constants and rate of loss of substituted anilines to a sediment sample for the initial rapid step, but for the second slow step, they reported a much weaker correlation. In contrast, Li et al. (36) reported a strong correlation between Hammett constants and irreversible binding to soil samples.

Anilines can form imines with $p$-benzoquinone via a reaction with water as a byproduct (37). The imine may be an important product in nonaqueous solution, but in aqueous solution imines are readily hydrolyzed back to the starting reactants. In literature reports (7, 8), the final isolatable product is reported to result from Michael addition. Although, it is not possible to distinguish imine products from Michael addition products without authentic standards, our experiments are conducted in aqueous solution over long time periods so predominantly Michael addition products are expected under these conditions.
Addition products and NERs can also result from radical addition mechanisms (1). Thomson (38) suggested that thiol compounds add to juglone via a radical addition mechanism. Later, Perlinger et al. (39) reported that Michael addition and radical addition were both active in the reaction of bisulfide and juglone to form mercapto substituted juglone. They reasoned that increasing pH favors the radical pathway because the radical pathway involves loss of a proton where Michael addition requires gain of a proton and EPR (electron paramagnetic resonance) detected increasing yield of semiquinone radical with increasing pH (39). In these studies, radical generation occurred via redox mechanisms. Our experimental solutions contain no added oxidants or reductants and are sparged with N₂(g) to minimize dissolved O₂ concentration. The intent of these measures is to mitigate redox reactions in our reaction solutions and suppress radical formation.

In this study, we examine loss of p-benzoquinone and patulin in the presence of model nucleophilic contaminants. We will also discuss complications associated with loss of p-benzoquinone or patulin in the absence of an added nucleophile, where we expect H₂O, OH⁻, and buffer compounds to be the most important potential nucleophiles. A series of substituted anilines are used to develop a Hammett relationship and explore the effect of electronic effect on rate of nucleophilic attack. Lastly, loss of p-benzoquinone in the presence of complicated nucleophiles such as aminopyralid, chloramben, and clopyralid is discussed as a means to assess nucleophilicity.

2.2 Materials and Methods

All aqueous solutions were prepared from reagent grade chemicals and distilled, deionized water (Milli-Q water, 18 M-cm resistivity, Millipore Corp., Milford, MA). All bottles
and glassware were rinsed with distilled water, soaked in 5 M nitric acid overnight, rinsed with distilled water and Milli-Q water and air-dried.

2.2.1 Chemicals

3-(N-Morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino)ethanesulfonic acid monohydrate (MES), sodium acetate, and ascorbic acid were purchased from Sigma (St. Louis, MO). p-Benzoquinone, 4-methylimidazole, aniline, maleic acid, N-cyclohexyl-2-aminoethanesulfonic acid (CHES), 4-aminophenol, 4-methoxyphenol, 4-ethylaniline, and ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) were purchased from Aldrich (St. Louis, MO). p-Benzoquinone was purified by sublimation before use. Patulin was purchased from A. G. Scientific (San Diego, CA). 4-Methoxyaniline (p-anisidine), 4′-aminoacetophenone, hydroxylamine hydrochloride, and 4-nitroaniline were purchased from Alfa Aesar (Pelham, NH). 1,4-Diethylpiperazine (DEPP), chloramben, (3-amino-2,5-dichlorobenzoic acid), and NaCl were purchased from Acros (Fair Lawn, NJ). HPLC grade methanol and HCl were purchased from Fisher Scientific (Pittsburgh, PA). Clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) and ammonium acetate were purchased from Fluka (Buchs, Switzerland). 1,2,4-Trihydroxybenzene was purchased from TCI America (Portland, OR). Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) was purchased from Chem Service (West Chester, PA). NaOH was purchased from J. T. Baker (Phillipsburg, NJ). 3-Hydroxypyridine was purchased from Lancaster Synthesis (Morecombe, UK).

2.2.2 Experimental Design

Stock solutions were prepared freshly each day. Stocks (10 mM) of Michael acceptor electrophile (patulin or p-benzoquinone) were prepared in methanol because of their low
aqueous solubility. Stock solutions of MOPS buffer, NaCl, and nucleophile of interest were prepared in Milli-Q water. The MOPS buffer stock was adjusted to pH 7.0 using NaOH and HCl. Sufficient volume of nucleophile, MOPS, NaCl, and Michael acceptor stocks were pipetted into a 25 mL volumetric flask to give a solution consisting of 100 µM Michael acceptor electrophile, 1.0 mM or 10 mM nucleophile, 5 mM MOPS, and 10 mM ionic strength when diluted to the mark. This solution was sparged with N₂(g) for 30 min to remove O₂. Then sufficient stock of Michael acceptor electrophile was added to initiate reaction, the solution was diluted to the mark, and the time of addition was recorded. These reaction solutions contained less than 1% methanol. A separate solution containing no added nucleophile served as a control. Working as quickly as possible following addition of the electrophile, aliquots of each solution were pipetted into separate autosampler vials. The vials were sealed with Teflon septa and loaded into an autosampler for HPLC analysis. Inside the autosampler, the samples were held in the dark and at room temperature (22 – 23°C). Each vial was injected into the HPLC only once and the time of injection was recorded. The time point for a sample on a time course plot was the difference between the time of reaction initiation and the time of injection. Most time courses lasted 4 hours. One exception is patulin loss time course data in the absence of an added nucleophile. These solutions were prepared as described above and placed in open polypropylene bottles in a constant temperature bath held at 25±1°C and were continuously sparged with N₂(g). Samples were withdrawn periodically transferred to autosampler vials that were then analyzed by HPLC.

2.2.3 Instrumentation and Analysis

*p*-Benzoquinone and patulin concentration was monitored using reversed-phase HPLC (Waters Corp., Milford, MA) run in isocratic mode with 85% 5 mM ammonium acetate and
15% methanol (by volume) at a flow rate of 1.0 mL min\(^{-1}\). The column was a Spherisorb ODS2 5\(\mu\)m 5.6 × 150 mm (Waters Corp., Milford, MA). UV detection was used for \(p\)-benzoquinone at 245 nm and at 280 nm for patulin. Visible light absorbance at 510 nm and spectra were recorded using a Shimadzu UV-1800 spectrophotometer with 1 cm quartz cuvettes.

LC/MS with electrospray ionization was used in an attempt to observe 1,2,4-trihydroxybenzene in experiments with \(p\)-benzoquinone. LC separation was conducted using a Waters 2795 separation module (Milford, MA) with a 4.6 x 100 mm, 5 \(\mu\)m particle size, Atlantis T3 column (Waters, Milford, MA) run in isocratic mode with 70\% 5 mM ammonium acetate and 30\% methanol at 200 \(\mu\)L min\(^{-1}\) as the eluent. LC/MS was able to detect authentic standards of 1,2,4-trihydroxybenzene to a detection limit of 5 \(\mu\)M, but this analyte was not detected in any experimental samples.

In our experimental solutions, the added nucleophile concentration is 10 times greater than the concentration of \(p\)-benzoquinone or patulin. Under these conditions, an observed rate constant \((k_{obs})\) can be obtained from fitting time course data to a pseudo-first-order model:

\[
\frac{d[Z]}{dt} = -k_{obs}[Z]
\]  

(2.3)

where \(Z\) is the Michael acceptor. Pseudo-first order conditions apply because the nucleophile is present in greater than order-of-magnitude excess of the Michael acceptor. Both slope and initial concentration in the integrated form of Equation 2.3 were used as fitting parameters. Second-order rate constants are calculated by normalizing the pseudo-first-order rate constants by the initial concentration of the nucleophile. These rate constants are normalized by the initial concentration of the deprotonated form of the nucleophile rather than the total
nucleophile concentration:

\[ k_{\text{Nu}^-} = \frac{k_{\text{obs}} K_a \text{Nu}_T}{K_a + [\text{H}^+]} \]  \hspace{1cm} (2.4)

where \( k_{\text{Nu}^-} \) is the corrected second-order rate constant, and \( K_a \) is the acid dissociation constant of the nucleophile.

Increase in absorbance at 510 nm over time will also be used to determine a pseudo-first-order rate constants. In this case, consider the concentration of the product of a \( Z \rightarrow P \) first-order reaction \((40, \text{p. 27})\)

\[ [P] = [P]_0 + [Z]_0 \left(1 - e^{-k_{\text{obs}}t}\right) \]  \hspace{1cm} (2.5)

where is \( P \) is the reaction product. Assuming that Beer’s law holds for \( P \) at 510 nm, the concentration of \( P \) is related to the absorbance \((abs_{510 \text{ nm}})\) by the extinction coefficient of \( P \) at 510 nm \((\epsilon_P)\).

\[ abs_{510 \text{ nm}} = \epsilon_P \ell [P] \]  \hspace{1cm} (2.6)

Combining Equations 2.5 and 2.6 gives

\[ abs_{510 \text{ nm}} = \epsilon_P \ell [P]_0 + \epsilon_P \ell [Z]_0 \left(1 - e^{-k_{\text{obs}}t}\right) \]  \hspace{1cm} (2.7)

Equation 2.7 can be used to determine pseudo-first-order rate constants for time course plots of 510 nm absorbance. The second-order rate constant of generation of a product of an added nucleophile and \( p \)-benzoquinone that absorbs at 510 nm was determined from a series of experiments with initial concentration of \( p \)-benzoquinone of 100 \( \mu \text{M} \) and increasing concentration of added nucleophile. The relationship between \( k_{\text{obs}} \) and the initial
concentration of added nucleophile \([\text{[Nu]}_0]\) is linear

\[
k_{\text{obs}} = k\text{[Nu]}_0
\]  \hspace{1cm} (2.8)

and the slope determined by linear regression gives the second order rate constant \((k)\).

### 2.3 Results and Discussion

#### 2.3.1 \(p\)-Benzoquinone

Time courses showing loss of \(p\)-benzoquinone time courses in the presence of 1 mM or 10 mM added nucleophiles are shown in Figure 2.3. Under the conditions of Figure 2.3, less than 5\% loss of \(p\)-benzoquinone was observed in the absence of added nucleophile. See Section 2.5.2 in the Supporting Information for more information on our preliminary studies of loss of \(p\)-Benzoquinone in pH buffered solutions that do not contain an added nucleophile. These preliminary studies may suggest that a general base catalysis mechanism is active in DEPP, MES, and MOPS solutions but the evidence in the Supporting Information is insufficient to unequivocally reach that determination. Observed pseudo-first-order rate constants and \(k_{\text{Nu}^-}\) values are shown in Table S2.1 in the supporting information.

Whenever we add a nucleophile to solutions of \(p\)-benzoquinone, the added nucleophile competes with \(\text{H}_2\text{O}, \text{OH}^-, \text{and buffer}\). The rate of reaction with the added nucleophile depends on how strong the nucleophile is and the concentration of its active, deprotonated form. Additionally, the added nucleophile must be strong enough and/or concentrated enough to outcompete other nucleophiles present in solution. In Figure 2.3, a high concentration (1 mM) of aniline and 4-methylimidazole readily outcompeted \(\text{H}_2\text{O}, \text{OH}^-\), and MOPS
buffer for reaction with \( p \)-benzoquinone. However, because 1mM 4-methoxyphenol did not result in significantly greater loss of \( p \)-benzoquinone than in the control, we increased the concentration to 10 mM.

If the rate of \( p \)-benzoquinone loss were simply a function of \( pK_a \), then we would expect 4-methoxyphenol would react more rapidly than 4-methylimidazole or aniline. However, we observed the opposite trend (Figure 2.3). At pH 7, 4-methoxyphenol is nearly 100% protonated, 4-methylimidazole is 77% protonated, and aniline is almost entirely protonated. The nucleophile with the highest proportion of deprotonated form leads to the fastest rate of loss of \( p \)-benzoquinone. The rate constants followed the same trend: \( 2.3 \times 10^{-1} \text{ M}^{-1} \text{s}^{-1} \) for aniline, \( 2.2 \times 10^{-3} \text{ M}^{-1} \text{s}^{-1} \) for 4-methylimidazole, and \( 7.06 \times 10^{-4} \text{ M}^{-1} \text{s}^{-1} \) for 4-methoxyphenol (Table S2.1). Based on these constants, aniline is a stronger nucleophile than 4-methylimidazole, which is stronger than 4-methoxyphenol.

We observed growth of an absorbance peak centered around 400 and 510 nm in UV/Vis spectra recorded in solutions of 100 \( \mu \text{M} \) \( p \)-benzoquinone and aniline at pH 7.0 in 5 mM MOPS buffer (Figure 2.4A). The 510 nm absorbance reached 0.4 absorbance units in 60 min in the absence of aniline (blank on Figure 2.4) which is could be attributed to hydration, but could also be due to adduct formation with MOPS. In the supporting information (section 2.5.2) we present results of preliminary experiments that may indicate adduct formation of MOPS. The expected hydration product of \( p \)-benzoquinone, 1,2,4-trihydroxybenzene, could not be detected by LC/MS. MOPS buffered solutions (5 mM at pH 7.0) of 1,2,4-trihydroxybenzene do quickly change color and exhibit absorbance at 510 nm (Figure S2.3 in the Supporting Information). The rate of increase of absorbance at 510 nm increased with increasing concentration of aniline. The concentration dependence of \( k_{obs} \) increased linearly with the initial concentration of aniline (Figure 2.4B). Pseudo-first-order rate constants were determined using Equation 2.7 and the second-order constant was obtained using 2.8. The
second-order rate constant was $3.8 \pm 0.810^{-1} \text{ M}^{-1} \text{ s}^{-1}$. Monitoring loss of $p$-benzoquinone yielded a similar but slightly smaller rate constant: $2.3 \times 10^{-1} \text{ M}^{-1} \text{ s}^{-1}$.

The rate constant determined from 510 nm absorbance (Figure 2.4) corresponds to only the generation of a $p$-benzoquinone and aniline reaction product if only that product absorbs at 510 nm. In the absence of aniline, we observed some absorbance at 510 nm in solutions of 100 µM $p$-benzoquinone in 5.0 mM MOPS (Figure S2.7) but less absorbance was observed than in solutions containing aniline (Figure 2.4). In the absence of $p$-benzoquinone, no absorbance is observed from solutions of aniline under the same conditions and timescale as Figure 2.4. Also, The linear relationship between observed rate constant and aniline concentration (Figure 2.4B) supports the conclusion that 510 nm absorbance comes from a reaction product of aniline.

### 2.3.2 Patulin

Time courses for loss of patulin in the presence of representative nucleophiles (para-substituted anilines, 4-methoxyphenol, and hydroxylamine) are shown in Figure 2.5. No significant loss of patulin was observed when 4-methylimidazole, 4-methoxyphenol, or 4-acetylphenol was added to serve as the nucleophile. No significant loss of patulin was observed in the absence of an added nucleophile in 5 mM MOPS at pH 7.0. Section 2.5.3 in the Supporting Information presents results of preliminary experiments of loss of patulin in solutions of varying pH and buffer.

### 2.3.3 Comparison of Electrophilicity: $p$-Benzoquinone and Patulin

The rate constants measured for reactions of nine nucleophiles with $p$-benzoquinone and six with patulin are summarized in Table 2.1. For nucleophiles where rate constants were
measured for both *p*-benzoquinone and patulin, the values are between 1 and 2 orders of magnitude larger for *p*-benzoquinone than for patulin.

For both *p*-benzoquinone and patulin the Hammett $\rho$ value determined from the slope of the Hammett plot was negative (Figure 2.6). The Hammett $\sigma$ values are taken from McDaniel et al. (41). The $\rho$ value was greater in magnitude for *p*-benzoquinone ($−2.1 ± 0.3$) than for patulin ($−1.2 ± 0.2$). Para-substituted anilines react with *p*-benzoquinone 1–2 orders of magnitude faster than with patulin (Figure 2.6).

Negative $\rho$ values mean that substituted anilines containing electron-donating substituents increase the rate of reaction relative to aniline. Electron-donating substituents increase electron density at the nucleophilic center atom making nucleophilic attack more favorable. Negative $\rho$ values also imply build-up of positive charge in the transition state, which is stabilized by increased electron density at the nucleophilic center. Both of these findings are consistent with the Michael addition mechanism where the nucleophile donates two electrons to the Michael acceptor (Figure 2.1). Table 2.2 compares the $\rho$ values obtained in this study with those available in the literature. Colón et al. (11) reported $\rho$ values from biphasic kinetics of loss of aromatic amines to sediment samples: the $\rho$ value was $−1.7$ for the “fast” first step and $−0.4$ for the “slow” second step. Our $\rho$ value for *p*-benzoquinone ($−2.1 ± 0.3$) is larger in magnitude, but supports the conclusion that Michael type nucleophilic addition is involved in irreversible binding of anilines. Parris (8) reported a Hammett $\rho$ value of $−1.5$ for *p*-benzoquinone reaction with 4-methylaniline, aniline, 4-chloroaniline, and 3,4-dichloroaniline. His reaction conditions differed slightly from those reported here: his solutions contained 71% methanol and no buffer (8), where the results reported here used less than 1% methanol and were buffered using 5 mM MOPS adjusted to pH 7.0. Solvent and pH effects may explain why our $\rho$ value is larger in magnitude than the value reported by Parris.
One explanation for the higher rate constants observed for \( p \)-benzoquinone than patulin (Table 2.1) is that nucleophiles react faster with \( p \)-benzoquinone than with patulin because of structural differences: \( p \)-benzoquinone undergoes 1,4-addition while patulin must undergo 1,6-addition. In 1,4-addition the electrophilic site is two atoms closer to the electronegative oxygen atom that ultimately accepts the electrons from the nucleophile. In 1,6-addition these electrons must traverse an additional carbon-carbon double bond. Electron density at electrophilic site in Michael acceptors is withdrawn by the oxygen atom, and should be withdrawn more strongly when it is two atoms away instead of four. Also, there are four possible Michael addition sites on \( p \)-benzoquinone where patulin has one. So, \( p \)-benzoquinone could be four times as likely to react with a nucleophile than patulin at the same concentration.

### 2.3.4 Assessing Nucleophilicity: Aminopyralid, Chloramben, and Clopyralid

In the previous sections, we were primarily interested in loss of the Michael acceptor in the presence of simple model nucleophiles that only contained one Lewis base group. We could assess and compare nucleophilicity of compounds based on observed \( p \)-benzoquinone loss rate at a given pH and buffer. This approach could also be valuable for complicated nucleophiles. Consider the rate of \( p \)-benzoquinone loss observed in the presence of 1 mM clopyralid, aminopyralid, or chloramben (Figure 2.7). These solutions were buffered with 5.0 mM MOPS adjusted to pH 7.0. Aminopyralid and clopyralid react at similar rates, with rate constants of \( 1.69 \times 10^{-3} \) and \( 1.84 \times 10^{-3} \) M\(^{-1}\) s\(^{-1}\), respectively. Chloramben reacted significantly faster, the rate constant was \( 1.2 \times 10^{-2} \) M\(^{-1}\) s\(^{-1}\).
The structures of the three herbicides differ in whether or not they include an aniline-like amine nitrogen or a pyridine-like heterocyclic nitrogen (Figure 2.2). These compounds are more complicated than our model nucleophiles because they contain multiple potential nucleophilic sites. Heterocyclic nitrogen atoms are not as strongly nucleophilic as aniline: 4-methylimidazole reacted much more slowly than aniline with p-benzoquinone (Figure 2.3) and pyridine was reported to form no covalent bonds with NOM (5).

Based on these simple experiments, chloramben is the most nucleophilic of the three herbicides that we tested. We anticipate that nucleophilicity measured this way will correlate with the rate of loss to irreversible binding to organic matter in soils or sediments. If this were true, then chloramben would be a more environmentally friendly pesticide choice than aminopyralid or clopyralid because it would quickly become biologically unavailable. Although making this conclusion is not possible with our current limited data set, future research work could explore the nature of the relationship between p-benzoquinone loss rates and rate of loss via irreversible binding to organic matter. This could inform both choice and design of environmentally friendly chemicals and development of fate and transport models that properly account for this loss mechanism.

2.4 Conclusions

We examined time course data for loss of p-benzoquinone and patulin Michael acceptor electrophiles in the presence of several added nucleophiles under pseudo-first-order conditions. Under these conditions, we tentatively attribute loss of p-benzoquinone and patulin primarily to Michael-type nucleophilic addition. Even in the absence of an added nucleophile, loss of p-benzoquinone and patulin was observed in buffered solutions, and is related to pH and structure of compound employed as the buffer. We urge readers to carefully
consider buffer choice when conducting experiments on nucleophile-electrophile reactions in aqueous solution. Although we could observe the sum effect of added nucleophile, hydration, and buffer reaction on loss of electrophilic substrate, determining individual rate constants for each process will most likely require time course data of the reaction products.

Nucleophilic addition reactions that occur in sediments, soil, and water treatment processes deserves mechanistic study. We expect that the effects molecular structure and pH exert on nucleophilicity, electrophilicity, and protonation state are key to determining the importance of these reaction pathways. We studied Michael addition reactions with p-benzoquinone in buffered 99% aqueous solutions to simulate the slow irreversible binding of nucleophiles to natural organic matter (42), which had previously been studied in mixed solvents (8). We determined that a Hammett relationship also exists for patulin, a natural toxic compound, that undergoes a more complex addition pathway than does p-benzoquinone (29, 43). As we become more aware of potential water quality impacts of mycotoxins arising from crop fields (25) we will need to consider the ramifications of electrophilic and nucleophilic properties on their environmental fate and transport. Lastly, we proposed that p-benzoquinone loss rate could be a simple tool to predict loss of any contaminants possessing a nucleophilic functional group such as the herbicides chloramben, aminopyralid and clopyralid.

References


Table 2.1. Second-order rate constants determined for reactions of added nucleophiles with *p*-benzoquinone and patulin Michael acceptor. Rate constants \(k_{\text{Nu}^-}\) corrected to reflect the deprotonated form of the nucleophile (Equation 2.4). Half-life calculated for 1 mM of the deprotonated form of the nucleophile.

<table>
<thead>
<tr>
<th>Michael Acceptor</th>
<th>Nucleophile</th>
<th>(k_{\text{Nu}^-}) (M(^{-1}) s(^{-1}))</th>
<th>Half-life (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>p</em>-Benzoquinone</td>
<td>Aminopyralid</td>
<td>(1.7 \times 10^{-3})</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>Clopyralid</td>
<td>(1.8 \times 10^{-3})</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>4-Nitroaniline</td>
<td>(6.2 \times 10^{-3})</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Chloramben</td>
<td>(1.2 \times 10^{-2})</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>4′-Aminoacetophenone</td>
<td>(1.8 \times 10^{-2})</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>3-Hydroxypyridine</td>
<td>(1.6 \times 10^{-1})</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>4-Methylimidazole</td>
<td>(2.2 \times 10^{-1})</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Aniline</td>
<td>(2.3 \times 10^{-1})</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>4-Ethylaniline</td>
<td>(5.4 \times 10^{-1})</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>4-Methoxyphenol</td>
<td>(1.14)</td>
<td>0.2</td>
</tr>
<tr>
<td>Patulin</td>
<td>4-Nitroaniline</td>
<td>(4.4 \times 10^{-4})</td>
<td>430</td>
</tr>
<tr>
<td></td>
<td>4′-Aminoacetophenone</td>
<td>(7.1 \times 10^{-4})</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>Aniline</td>
<td>(3.0 \times 10^{-3})</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Hydroxylamine</td>
<td>(4.1 \times 10^{-3})</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>4-Methoxyaniline</td>
<td>(8.0 \times 10^{-3})</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>4-Aminophenol</td>
<td>(9.8 \times 10^{-3})</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>4-Methoxyphenol</td>
<td>(4.1 \times 10^{-2})</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 2.2. Hammett reaction constants ($\rho$) for para-substituted anilines determined in this study and available in the literature.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Substrate</th>
<th>$\rho$ ($\sigma$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>This Study</td>
<td>$p$-Benzoquinone$^a$</td>
<td>−2.1</td>
</tr>
<tr>
<td>This Study</td>
<td>Patulin</td>
<td>−1.2</td>
</tr>
<tr>
<td>Parris 1980 (8)</td>
<td>$p$-Benzoquinone$^b$</td>
<td>−1.5</td>
</tr>
<tr>
<td>Iskander et al. 1988 (35)</td>
<td>$p$-Benzoquinone$^c$</td>
<td>−1.4</td>
</tr>
<tr>
<td>Li et al. 2000 (36)</td>
<td>Fast Step</td>
<td>−1.3</td>
</tr>
<tr>
<td></td>
<td>Slow Step</td>
<td>−1.25</td>
</tr>
<tr>
<td>Colón et al. 2002 (11)</td>
<td>Fast Step</td>
<td>−1.75</td>
</tr>
<tr>
<td></td>
<td>Slow Step</td>
<td>−0.45</td>
</tr>
</tbody>
</table>

$^a$ in aqueous solution containing < 1% methanol.  
$^b$ in methanol.  
$^c$ in ethanol.
Figure 2.1. Proposed mechanism for Michael addition of a nucleophile (Nu-) to \( p \)-benzoquinone.
Buffers

- DEPP
- MES
- MOPS
- CHES

Electrophilic Substrates

- $p$-benzoquinone
- patulin
- juglone

Added Nucleophiles

- aromatic amines
- 4-methylimidazole
- 3-hydroxypyridine
- 4-methoxyphenol

Nucleophilic Contaminants

- chloramben
- aminopyralid
- clopyralid

Figure 2.2. Structures of organic compounds discussed in this study.
Figure 2.3. *p*-Benzoquinone loss in the presence of an added nucleophile: (A) approximately 1 mM added nucleophile, and (B) approximately 10 mM added nucleophile. Conditions: 100 μM *p*-benzoquinone, 5 mM MOPS adjusted to pH 7.0 and 10 mM NaCl. The blank contains no added nucleophile.
Figure 2.4. (A) Increase in absorbance at 510 nm absorbance in a solution containing 100 µM $p$-benzoquinone and aniline. Conditions: 5.2 mM MOPS buffer adjusted to pH 7.0, 10 mM NaCl. (B) Observed rate constants ($k_{obs}$) determined from increase in 510 nm absorbance.
Figure 2.5. Patulin loss in the presence of an added nucleophile. Conditions: 100 µM p-benzoquinone, 1 mM added nucleophile, 5 mM MOPS adjusted to pH 7.0 and 10 mM NaCl.
Figure 2.6. Correlation of Hammett constant ($\sigma$) and rate constant ($k_{Nu^-}$) for loss of $p$-benzoquinone or patulin in the presence of monosubstituted anilines. The substituent is marked for each point. The slope, $\rho$, for $p$-benzoquinone is $-2.1 \pm 0.3$. For patulin the slope is $-1.2 \pm 0.2$. Rate constants are reported in Table 2.1.
Figure 2.7. $p$-Benzoquinone consumption in the presence of aminopyralid, clopyralid, or chloramben: measured $p$-benzoquinone concentration ($C$) normalized by initial $p$-benzoquinone concentration ($C_0$). Conditions: 100 µM $p$-benzoquinone, 1 mM aminopyralid, clopyralid, or chloramben in 5.0 mM MOPS buffer adjusted to pH 7.0.
2.5 Supporting Information

Supporting information for Chapter 2 contains a description of the nucleophilic addition to patulin mechanism proposed by Fliege and Metzler (1, 2), apparent rate constants for loss of p-benzoquinone in the presence of nucleophiles (Table S2.1), and for patulin (Table S2.2), 510 nm absorbance in solutions of p-benzoquinone and DEPP (Figure S2.5), MES (Figure S2.6) and MOPS (Figure S2.7), and a table of available acid dissociation constants (pK_a values) for chemical compounds used in this chapter.

2.5.1 Patulin Michael Addition Mechanism Proposed by Fliege and Metzler

Fliege and Metzler (1, 2) observed Michael addition reactions of patulin (Figure 2.2) and excess concentrations of glutathione, N-acetyl-L-cysteine, p-bromothiophenol, ethanolamine, mercaptoethanol, and bromoaniline nucleophiles during incubations in 50 mM or 1M phosphate buffered (pH 7) water-methanol solutions. All nucleophiles reacted with patulin to form a short-lived monoadduct. For bromothiophenol, the first addition occurs at C-6. They isolated the resulting adduct and confirmed its structure using ¹H and ¹³C NMR (2). Glutathione, N-acetyl-L-cysteine, and mercaptoethanol produced monoadducts where addition occurred at C-6 or C-2. The monoadducts transform via rearrangements and eliminations to form intermediates containing electrophilic α,β-unsaturated carbonyl substructures. The hemiacetal (C-4) can tautomerize to an electrophilic aldehyde. Another nucleophilic addition step can occur, potentially leading to a second round of rearrangements and a third addition step. Ultimately, Fliege and Metzler observed four types of products: lactones, thioenolether ketones, dihydropyranones, and ketohexanoic acids. NAC and GSH
formed thioenolether ketones at C-2 and C-6, but lactones were not observed; they were ei-
ther too short lived or lacked strong UV chromophores required to be detected by UV/HPLC.
Methyl ester products were reported arising from methanolysis of lactone rings in the pres-
ence of methanol solvent. The dihydropyranone products do not add another nucleophile
despite containing an \( \alpha,\beta \)-unsaturated carbonyl backbone. Fliege and Metzler reasoned that
the ring oxygen suppresses its electrophilicity and deactivates it. 4-Bromoaniline reacted
more slowly than the other nucleophiles tested. Both mono and diadducts were identified.

Fliege and Metzler reported that 1, 2, and 3 were the identifiable products of the reaction
between aniline and patulin (1, 2). The following is a more detailed description of the
mechanism that they proposed. These products arise from nucleophilic attack by aniline
or water. There are two nucleophilic attack steps in the proposed mechanism. \( \text{Nu}_1 \) is
represents the nucleophile that attacks first (either aniline or water) and \( \text{Nu}_2 \) represent the
second nucleophile. In this case, \( \text{Nu}_1 \) or \( \text{Nu}_2 \) in the products (1, 2, and 3) represents either a
hydroxyl group or aniline.

The first step is Michael-type 1,6 addition to C-6. The resulting zwitterion undergoes
proton exchange. The enol then tautomerizes to the more stable keto form.
Lactones are susceptible to hydrolysis. The hydrolysis product undergoes keto-enol tautomerization to the more stable keto form. This reaction can be base catalyzed:

This step could also be acid catalyzed, following protonation of the carbonyl oxygen:

In the next step, a six-member transition state allows proton transfer from the hemiacetal oxygen to the keto oxygen, opening the hemiacetal ring. The ketone reforms through keto-enol tautomerization.
C-4 can now be eliminated by an E2 mechanism. The leaving group is a formate ion consisting of C-4 and two adjoining oxygens.

\[ \text{Nu} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{H} \quad \text{O} \quad \text{O} \quad \text{H} \quad \text{H} \quad - \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{H} \quad \text{O} \quad \text{O} \quad \text{H} \quad \text{H} \quad (S2.5) \]

The elimination product is an \( \alpha, \beta \)-unsaturated carbonyl. Michael-type 1,4-addition of a second nucleophile (Nu\(_2\)) and a keto-enol tautomerization leads to the products (1, 2, and 3).

\[ \text{HNu}_1 \quad \text{Nu}_1 \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{H} \quad \text{O} \quad \text{O} \quad \text{H} \quad \text{H} \quad \text{HNu}_2 \quad \text{O} \quad \text{O} \quad \text{H} \quad \text{O} \quad \text{O} \quad \text{H} \quad \text{H} \quad \text{HNu}_2 \quad \text{O} \quad \text{O} \quad \text{H} \quad \text{O} \quad \text{O} \quad \text{H} \quad \text{H} \quad (S2.6) \]

### 2.5.2 \( p \)-Benzoquinone and Reactions with Buffer Compounds

Absorbance of solutions of \( p \)-benzoquinone and aniline at 510 nm has been attributed to the formation of the Michael-type adduct: anilinohydroquinone (3). However, in the absence of aniline, some 510 nm absorbance was observed after 2 hours of contact time in buffered 1 mM \( p \)-benzoquinone solutions, regardless of whether the buffer was MOPS, phosphate, or maleate at pH 7.0 (Figure S2.2). At pH 2.0, no significant absorbance was observed above 500 nm, regardless of buffer identity. Both phosphate and maleate have buffer capacity near pH 2.0, their pK\(_a\) values are 2.148 and 1.92, respectively (25°C and zero ionic strength, 4). MOPS does not have a pK\(_a\) near pH 2, but the solution pH did not change significantly in 2 hours. At pH 7 and pH 2, MOPS buffered solutions exhibited higher absorbance than solutions of the other buffers. At 5 mM buffer concentration, 510 nm absorbance was 2 times higher in MOPS solution than in phosphate or maleate solutions. Increasing the buffer
concentration by an order of magnitude strongly increased absorbance of MOPS solutions, somewhat for phosphate, but only slightly for maleate: 510 nm absorbance was 3 times higher for 50 mM MOPS than 5.0 mM MOPS, 1.76 times higher for 50 mM phosphate versus 5 mM phosphate, and 1.24 times higher for 50 mM maleate versus 5.0 mM maleate.

Tentatively, we can attribute the absorbance observed above 500 nm in maleate solution to products of hydration, and not products arising from addition of maleate. If this absorbance indicated a maleate addition product then increasing the maleate concentration by an order-of-magnitude we expect that the absorbance at 500 nm would have greatly increased. However, the increase in absorbance above 500 nm with increased maleate concentration close to insignificant. Instead of a maleate addition product, we attribute this to a hydration product. In contrast, increased MOPS concentration lead to a much higher absorbance, suggesting that a product that absorbs at 510 nm is formed by reaction of MOPS and $p$-benzoquinone.

The spectrum of the expected hydration product, 1,2,4-trihydroxybenzene is shown in Figure S2.3. A solution of 1,2,4-trihydroxybenzene in 5.0 mM MOPS buffer adjusted to pH 7.0 rapidly changed color before the spectrum could be recorded. One potential explanation for the color change is oxidation of 1,2,4-trihydroxybenzene to 2-hydroxybenzoquinone by $O_2$. Either this benzoquinone or subsequent polymerization products may cause the absorbance observed around 500 nm. In methanol, where we do not expect significant $O_2$ oxidation, no peak around 500 nm is observed (Figure S2.3).

Near the $p^aK_a$ of MOPS (7.184, 4), solution pH strongly affected absorbance spectra of solutions containing 1 mM $p$-benzoquinone and 5.0 mM MOPS (Figure S2.4). Absorbance at 510 nm increased from pH 6.2 to 7.8. The spectrum taken at pH 5.5, outside the buffer range of MOPS, has much lower absorbance above 500 nm and is similar to the spectrum observed at pH 2.0.
Assuming that the absorbance at 510 nm is due to a MOPS reaction product, the relationship between absorbance and pH near the pK\textsubscript{a} of MOPS reflects the influence of protonation. At pH 7.8, 80% of the MOPS in solution is deprotonated and the highest absorbance is observed. At pH 6.2, only 10% of the MOPS is deprotonated and the absorbance is considerably lower. Protonation of MOPS and nucleophilic attack by MOPS occurs at the same atom, likely the tertiary nitrogen, blocking the nucleophilic center by protonation and diminishing its ability to react with electrophiles.

We performed a series of time course experiments observing increase in absorbance at 510 nm of p-benzoquinone solutions buffered by DEPP at pH 4.6 (Figure S2.5), MES at pH 6.2 (Figure S2.6 and MOPS at pH 7.0 (Figure S2.7) with increasing concentration of buffer. For all three buffers, solutions of p-benzoquinone and MOPS at pH 7.0 (near the pK\textsubscript{a} of MOPS) the 510 absorbance slowly increased over time. Also, increasing the concentration of MOPS or MES increased the rate of absorbance increase. Compared to Figure 2.4 higher concentrations of p-benzoquinone and MOPS were required to yield the same absorbance that was observed with p-benzoquinone and aniline: 1 mM p-benzoquinone and 30 mM MOPS yield and absorbance of 0.14 after 70 min, where only 100 µM p-benzoquinone and 3 mM aniline are required to yield the same absorbance after 60 min (note that 5 mM MOPS was also present). However, this effect could be due to differences in the rate of adduct formation or differences in the molar absorptivity of the adducts. Authentic standards are not available for these adducts so we cannot determine their molar absorptivity. We speculate that these adducts would be hydroquinones with one hydrogen replaced with a C-N bond linking it to a molecule of buffer. In 5 mM MOPS (pH 7), only negligible absorbance at 510 nm was observed from 100 µM p-benzoquinone in the absence of aniline. MES solutions at pH 6.2 gave the same trend as MOPS, but the observed absorbance was lower in the same time frame (Figure S2.6). Solutions buffered using DEPP at pH 4.6 exhibited increases of
absorbance at 510 nm, but the increase was approximately 4 times slower than with MES (Figure S2.5).

MOPS, MES, and DEPP concentration dependence of the rate of increase in 510 nm absorbance suggests that at least some of this absorbance is due to a reaction product between these compounds and \( p \)-benzoquinone. Some absorbance is likely contributed by products that form by a parallel pathway. Importantly, to achieve the same absorbance the concentration of both \( p \)-benzoquinone and MOPS needs to be 10 times greater than when aniline is present: the contribution of a potential MOPS adduct or hydration product is an order of magnitude lower than that of the aniline adduct. Still, reactions of MOPS, MES, and DEPP with \( p \)-benzoquinone do complicate the interpretation of our data more than if the buffer compounds we used had been truly nonnucleophilic. Future researchers should be wary of these potential pitfalls and choose compounds to use as pH buffers wisely.

We expect that the observed rate of \( p \)-benzoquinone loss depends on (1) the pH, which controls [OH\(^-\)] and speciation of the buffer; and (2) the identity and concentration of the buffer. Both hydration and addition of buffer compounds occur simultaneously so it is only possible to discuss them in aggregate when considering loss of \( p \)-benzoquinone (Figure S2.8). DEPP and MES were used at 5 mM concentration to buffer pH in the range of 4 – 7. The half-life for loss of \( p \)-benzoquinone at these pH ranged from 74 to 250 hours, which corresponds to less than 10% loss of \( p \)-benzoquinone after 10 hours. The observed pseudo-first-order rate constant did not exceed \( 3 \times 10^{-6} \) s\(^{-1}\). At pH 7.02 in 5.0 mM MOPS buffer the half-life was 41 hours, which corresponds to 15% loss after 10 hours. At higher pH, loss of \( p \)-benzoquinone in 5.0 mM MOPS solution is more significant: at pH 7.6 and pH 8.1 the half-lives were 21 and 4 hours, respectively. In solutions buffered at pH 8.5-9.5 using 5 mM CHES, loss of \( p \)-benzoquinone was too fast to measure. Pseudo-first-order rate constants (\( k_{obs} \)) determined from the time course shown in Figure S2.8 are plotted as
a function of pH in Figure S2.9. The pH dependence of loss rate is readily apparent in Figure S2.9. In DEPP or MES solutions below pH 6.5 the $k_{obs}$ is smaller than $5 \times 10^{-6}$. For MOPS, this is consistent with Figure S2.4: $p$-benzoquinone loss and absorbance increase. Perhaps this is due to adduct formation that is faster at pH higher than the $pK_a$ of MOPS than at pH below it. At pH 4 — 6.5, much slower loss of $p$-benzoquinone indicates that (1) MES and DEPP react slowly with $p$-benzoquinone, (2) [OH$^-$] is too small to cause significant hydration, (3) the rate contribution of water is less than $3 \times 10^{-6}$ s$^{-1}$, and (4) and the product of rate constant and concentration of H$^+$ is smaller than $3 \times 10^{-6}$ s$^{-1}$ at these pH.

There is still doubt about the identity of the product that absorbs at 510 nm in the above experiments, and what mechanism leads to increase of absorbance at 510 nm. The absorbance increase could be due to the formation of an adduct between one of the compounds used as a buffer. However, a general base catalysis mechanism could also be active in which DEPP, MES, or MOPS deprotonation of a water molecule and nucleophile attack from that water molecule to $p$-benzoquinone both occur in the rate determining step. Unfortunately, insufficient evidence currently exists to reach either conclusion.

### 2.5.3 Patulin and Reactions with Buffer Compounds

Patulin is stable in buffered solutions lacking an added nucleophile in buffered solutions consisting of 5.0 mM acetate at pH 5.0, 5.0 mM MES at pH 6.0, and 5.0 mM MOPS at pH 7.0 (Figure S2.10). The slight increase in patulin concentration observed at pH 5.0, 6.0, and 7.0 is most likely due to evaporation of the solution. At pH 9.0 there was significant loss of patulin (Figure S2.10). This time course fits a pseudo-first-order model (Equation 2.3) with a measured $k_{obs}$ of $2.46 \times 10^{-5}$ s$^{-1}$. The data for pH 9.0 in Figure S2.10 yield a
$k_{\text{OH}^-}$ of 2.35 M$^{-1}$ s$^{-1}$. However, 5 mM CHES was also present to buffer the solution at pH 9.0. CHES contains a secondary amine functional group that could react with patulin as a nucleophile (Figure 2.2). Unfortunately, it is not possible with these data to tell how much of the loss of patulin was due to reaction with OH$, CHES, another solution constituent, or by oxidation by O$_2$ which may be significant at this pH.
Table S2.1. Pseudo-first-order rate constants observed for $p$-benzoquinone loss in the presence of nucleophiles ($k_{\text{obs}}$). Second order rate constants ($k$) determined by normalizing $k_{\text{obs}}$ by concentration of deprotonated nucleophile (Equation 2.4). Error shown determined from 95% confidence intervals for the slope of the best-fit line. Conditions: 5 mM MOPS and 10 mM NaCl. pK$_a$ values from Table S2.3. Hammett $\sigma$ constants (7) shown for aromatic amines.

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>[Nucleophile] (mM)</th>
<th>pH</th>
<th>pK$_a$</th>
<th>$\sigma$</th>
<th>$k_{\text{obs}}$ (s$^{-1}$)</th>
<th>$k$ (M$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>7.06</td>
<td></td>
<td></td>
<td>$3.4 \pm 0.5 \times 10^{-6}$</td>
<td></td>
</tr>
<tr>
<td>4-Ethylaniline</td>
<td>1.01</td>
<td>6.94</td>
<td>5.00</td>
<td>-0.151</td>
<td>$5.0 \pm 0.1 \times 10^{-4}$</td>
<td>$5.0 \pm 0.1 \times 10^{-1}$</td>
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<tr>
<td>Aniline</td>
<td>1.09</td>
<td>7.04</td>
<td>4.58</td>
<td>0</td>
<td>$2.5 \pm 0.1 \times 10^{-4}$</td>
<td>$2.3 \pm 0.1 \times 10^{-1}$</td>
</tr>
<tr>
<td>3-Hydroxypyridine</td>
<td>1.81</td>
<td>6.98</td>
<td>5.10</td>
<td>0</td>
<td>$2.5 \pm 0.1 \times 10^{-5}$</td>
<td>$1.38 \pm 0.05 \times 10^{-1}$</td>
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<tr>
<td>4'-Aminoacetophenone</td>
<td>1.18</td>
<td>6.99</td>
<td>2.75</td>
<td>0.502</td>
<td>$2.05 \pm 0.04 \times 10^{-5}$</td>
<td>$1.74 \pm 0.03 \times 10^{-2}$</td>
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<tr>
<td>4-Nitroaniline</td>
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<td>6.94</td>
<td>1.0</td>
<td>0.778</td>
<td>$6.8 \pm 0.4 \times 10^{-6}$</td>
<td>$5.8 \pm 0.4 \times 10^{-3}$</td>
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<td>4-Methylimidazole</td>
<td>10.87</td>
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<td>$2.4 \pm 0.1 \times 10^{-5}$</td>
<td>$2.2 \pm 0.1 \times 10^{-3}$</td>
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<tr>
<td>4-Methoxyphenol</td>
<td>12.18</td>
<td>7.02</td>
<td>10.21</td>
<td></td>
<td>$9.3 \pm 0.8 \times 10^{-6}$</td>
<td>$7.6 \pm 0.6 \times 10^{-4}$</td>
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Table S2.2. Pseudo-first-order rate constants observed for patulin loss in the presence of nucleophiles (k_{obs}). Second order rate constants (k) determined by normalizing k_{obs} by concentration of deprotonated nucleophile (Equation 2.4). Error shown determined from 95% confidence intervals for the slope of the best-fit line. Conditions: 5 mM MOPS and 10 mM NaCl. References for pK_{a} values given in Table S2.3. Hammett σ constants (7) shown for aromatic amines.

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>[Nucleophile]</th>
<th>pH</th>
<th>pK_{a}</th>
<th>σ</th>
<th>k_{obs} (s^{-1})</th>
<th>k  (M^{-1} s^{-1})</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>7.06</td>
<td></td>
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<td>2 ± 4 × 10^{-7}</td>
<td></td>
</tr>
<tr>
<td>4-Methoxyphenol</td>
<td>10.24</td>
<td>7.02</td>
<td>10.21</td>
<td></td>
<td>2 ± 3 × 10^{-7}</td>
<td>4 ± 5 × 10^{-2}</td>
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<tr>
<td>4-Aminophenol</td>
<td>9.98</td>
<td>7.16</td>
<td>5.64</td>
<td>−0.37</td>
<td>9.6 ± 0.3 × 10^{-5}</td>
<td>9.8 ± 0.3 × 10^{-3}</td>
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<td>4-Methoxyaniline</td>
<td>8.77</td>
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<td>−0.268</td>
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<td>Hydroxylamine</td>
<td>11.05</td>
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<td>4.07 ± 0.07 × 10^{-3}</td>
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<td>Aniline</td>
<td>10.10</td>
<td>7.12</td>
<td>4.58</td>
<td></td>
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<td>2.97 ± 0.07 × 10^{-3}</td>
</tr>
<tr>
<td>4'-Aminoacetophenone</td>
<td>10.06</td>
<td>7.02</td>
<td>2.75</td>
<td>0.502</td>
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<td>7.1 ± 0.7 × 10^{-4}</td>
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<tr>
<td>4-Nitroaniline</td>
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<td>1.0</td>
<td>0.778</td>
<td>1.05 ± 0.05 × 10^{-6}</td>
<td>4 ± 1 × 10^{-4}</td>
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Table S2.3. Selected pKₐ values of chemicals discussed in this chapter.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>pKₐ</th>
<th>s</th>
<th>Reference</th>
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<tr>
<td>Hydroxylamine</td>
<td>5.96</td>
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<td>8</td>
</tr>
<tr>
<td></td>
<td>13.74</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>4.756</td>
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<td></td>
</tr>
<tr>
<td>DEPP</td>
<td>4.67</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.83</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>MES</td>
<td>6.270</td>
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<td></td>
</tr>
<tr>
<td>MOPS</td>
<td>7.184</td>
<td>4</td>
<td></td>
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<tr>
<td>CHES</td>
<td>9.384</td>
<td>4</td>
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<tr>
<td>H₃PO₄</td>
<td>2.148</td>
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</tr>
<tr>
<td></td>
<td>7.198</td>
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</tr>
<tr>
<td></td>
<td>12.35</td>
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</tr>
<tr>
<td>Maleate</td>
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<td></td>
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<td>4</td>
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<tr>
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<td>10</td>
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<tr>
<td>Clopyralid</td>
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<td>11</td>
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<td>Aminopyralid</td>
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<td>4-Nitroaniline</td>
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<td>4-Methoxyphenol</td>
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<tr>
<td>4-Methylimidazole</td>
<td>7.54</td>
<td>8</td>
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</table>

ᵃ Acid dissociation constants were corrected to zero ionic strength using the Davies equation (14), when necessary. ᵇ Ionic strength not reported.
Figure S2.1. Proposed reaction scheme for reaction of patulin with 4-bromoaniline (Br-Ph-NH₂). Adapted from reference 1.
Figure S2.2. Spectra of 1 mM \( p \)-benzoquinone solutions recorded 2 hours after addition to each medium. Solutions also included 10 mM NaCl.
Figure S2.3. Spectra of 250 µM 1,2,4-trihydroxybenzene. In MOPS solution, the spectrum is taken following a rapid color change that occurred despite N₂ sparging.
Figure S2.4. Effect of pH on spectra of 1.0 mM $p$-benzoquinone collected 1 hour after addition to pH adjusted solution of 5.0 mM MOPS and 10 mM NaCl.
Figure S2.5. 510 nm absorbance increase of 1 mM \( p \)-benzoquinone solution as a function of DEPP concentration. Solutions were adjusted to pH 4.6 and also contained 10 mM NaCl.
**Figure S2.6.** Increase in 510 nm absorbance of 1 mM $p$-benzoquinone solutions as a function of MES concentration. Solutions were adjusted to pH 6.2 and also contained 10 mM NaCl.
Figure S2.7. Increase in 510 nm absorbance of 1 mM \( p \)-benzoquinone solutions as a function of MOPS concentration. Solutions were adjusted to pH 7.0 and also contained 10 mM NaCl.
Figure S2.8. $p$-Benzoquinone loss in the absence of added nucleophile. DEPP, MES, and MOPS were employed as buffers. Conditions: 100 µM $p$-benzoquinone, 5 mM buffer, 10 mM NaCl.
Figure S2.9. Pseudo-first-order rate constants for loss of \( p \)-benzoquinone calculated using the data in Figure S2.8.
Figure S2.10. Time course for patulin loss in four different buffer systems. Conditions: 100 µM patulin, 5 mM buffer, 10 mM NaCl.
References


Chapter 3

Highly Reactive $p$-Benzoquinone Electrophiles Generated by the Oxidation of Substituted Hydroquinones Using $\text{MnO}_2(\text{s, pyrolusite})$ as an Oxidant

3.1 Introduction

In Chapter 2, we discussed nucleophilic addition reactions of phenol and aniline compounds with $p$-benzoquinone which is an experimentally convenient electrophile because it is stable enough to use as a reagent in experimental solutions that include an added nucleophile of interest. In contrast, when a carboxyl group is present on the $p$-benzoquinone ring, the compound is so electrophilic it cannot be readily isolated and used as a substrate in experiments like those in Chapter 2. In fact, carboxy-$p$-benzoquinone can only be isolated in anhydrous chloroform because it is quickly degraded in aqueous solutions or as a neat solid (1).
However, carboxy-\(p\)-benzoquinone can be generated via oxidation of 2,4-dihydroxybenzoic acid:

\[
\begin{align*}
\text{Z} + 2 \text{H}^+ + 2 \text{e}^- & \iff \text{XH}_2 \\
\end{align*}
\]

Collectively we refer to this family of compounds as “quinones,” following the convention of Uchimiya and Stone (2, 3). We will refer to the hydroquinone form as \(\text{XH}_2\) and the oxidized form as the benzoquinone or \(\text{Z}\) regardless of the presence of substituents. \(\text{XH}_2\) are “proelectrophiles” which are transformed into electrophiles when they become oxidized. Oxidation of \(\text{XH}_2\) can be achieved enzymatically consuming \(\text{H}_2\text{O}_2\) or \(\text{O}_2\) (4–6), electrochemically (7, 8), or by reaction with \(\text{Mn}^{\text{III,IV}}\) (hydr)oxides (4).

In this work, we employ model proelectrophiles as substrates to mimic proelectrophile functional groups with electron withdrawing group substituents within natural organic matter (NOM). Hydroquinone and catechol moieties present in NOM could behave as proelectrophiles and become electrophilic following oxidation. NOM is thought to contain quinone functional groups that are active in reduction and oxidation reactions (9–11). Ratasuk et al. (12) divided quinone groups in NOM into two fractions: one that has electron-withdrawing substituents and one that has electron donating group substituents or is sterically hindered. We hypothesize that highly reactive electrophiles could be generated in soils and sediments from small molecule proelectrophiles like 2,4-dihydroxybenzoic acid or electron-withdrawing group substituted quinone groups in NOM. The electrophilic form could then react with nucleophiles, and potentially be an important removal mechanism for nucleophilic contaminants in the water column.
Several authors have reported that addition reactions of nucleophilic contaminants, particularly anilines, renders them biologically inactive by forming covalent bonds with natural organic matter in soils and sediments (4, 13–22). Weber et al. (14, 16) observed covalent binding of aniline to humic substances (16), and confirmed covalent bond formation using $^{15}$N NMR (14). Similar studies have reported irreversible sorption of aniline and $\alpha$-naphthylamine to soils either directly (23) or enhanced by oxidation of organic matter by manganese present in soils accompanied by an increase in exchangeable Mn$^{2+}$ (24, 25). Bialk et al. (4, 17) used $^{15}$N NMR to show that nucleophilic addition between sulfonamide antimicrobials and humic substances occurred via oxidation reactions caused by the manganese oxide solid birnessite and catalyzed by phenoloxidase enzymes.

Once an electrophile (Z) is generated it can be consumed by the same pathways discussed for $p$-benzoquinone in Chapter 2. Z can react with an added nucleophile to form an adduct (X-Nu). In aqueous solution, OH$^-$ or water can act as a nucleophile and react with Z; we call this “hydration” to distinguish it from reaction with non-solvent nucleophiles. Added nucleophiles compete with H$_2$O or OH$^-$ to react with benzoquinones (acid catalyzed hydration is not expected; 26). Hydration occurs via the same mechanism shown in Figure 2.1.

When XH$_2$ proelectrophiles are oxidized in the presence of nucleophiles the resulting adducts (X-Nu and X-OH) are themselves substituted hydroquinones. X-Nu and X-OH and can be oxidized to benzoquinones (Z-Nu and Z-OH). Z-Nu and Z-OH are second generation electrophiles because they result from a second oxidation step. For an XH$_2$ with two substituents, a third generation of Z can also be generated (Figure 3.1). Another round of addition/hydration–oxidation reactions is possible when the proelectrophile XH$_2$ has only 1 substituent. Figure 3.2 shows the sequence of reactions that leads to triadducts and fourth generation benzoquinones that are possible for monosubstituted XH$_2$ proelectrophiles. In this case, there are 52 possible products (Figure S3.3 in the Supporting Information).
Multiple addition products arising from sequential oxidation and addition steps have been reported for glutathione and \( p \)-benzoquinone (27) and glutathione with 2-bromohydroquinone (28). Glutathione adducts represent a detoxification pathway for quinone compounds, but they can also contribute toxicity, especially in the liver, by causing redox cycles that generate reactive oxygen species (29).

Generation of monoadduct and diadducts has been observed as new peaks in cyclic voltammograms when catechol and hydroquinone proelectrophiles are electrochemically oxidized in the presence of nucleophiles (30–33).

Replacing ring substituents with more electron-withdrawing substituents yields \( \text{XH}_2 \) with higher reduction potentials making them more difficult to oxidize (Table 3.1). A carboxylic acid substituent gives \( \text{XH}_2(\text{III}) \) a reduction potential of 0.769 V (34) which is 0.07 V greater than \( \text{XH}_2(\text{I}) \) which has a reduction potential of 0.699 V (35). Ring Substituents also influence electrophilicity of the benzoquinone (Z form). Electron-withdrawing substituents increase the rate of nucleophilic addition by increasing electron deficiency of the benzoquinone, while electron-donating substituents have the opposite effect (26, 31). Nucleophilicity is also subject to substituent effects: electron-donating substituents increase nucleophilicity and rates of nucleophilic reactions and electron-withdrawing substituents result in a decrease (31, 36). Lastly, regioselectivity is controlled by electronic effects of substituents. \( p \)-Benzoquinones with electron-withdrawing ring substituents preferably form adducts ortho to the substituent, while electron donating ring substituents favor adducts para to the substituent (33, 37). The electron-withdrawing or donating properties of ring substituents will also tune \( pK_a \) values for a given compound.

2,5-Dihydroxybenzoic acid (\( \text{XH}_2(\text{III}) \)), also known as gentisic acid, is an example hydroquinone proelectrophile possessing an electron-withdrawing carboxylic acid substituent. \( \text{XH}_2(\text{III}) \) is biosynthesized and by some plants and exuded into the soil near the plant.
roots (38). Metabolic degradation of carbamate and polycyclic aromatic hydrocarbons can also results in $XH_2(II)$ in groundwater (39).

Papouchado et al. (40, 41) reported hydroxylation products of EWG substituted hydroquinones that were oxidized electrochemically in an 0.10 M HClO$_4$ medium during cyclic voltammetry. The solvent, H$_2$O, is the only relevant nucleophile under these conditions because ClO$_4^-$ is a poor nucleophile and the OH$^-$ concentration is $10^{-13}$ M at this pH. The hydroxylated adduct does become oxidized under their conditions, but they noted that the electron-donating nature of the -OH group in the hydroxylated product prevents further hydroxylation reactions from occurring: no reaction of the 2$^{nd}$ generation benzoquinone was observed. Under our experimental conditions we expect that hydration products such as those detected by Papouchado and colleagues will be generated along with addition products with nucleophiles intentionall added to experiments. Also, because no second addition product was detected, their results confirm that EDG substituents, in this case $-OH$, decreases electrophilicity of 2$^{nd}$ generation benzoquinones relative to the 1$^{st}$ generation.

In our experiments, we employed a Mn$^{III,IV}$ (hydr)oxide as the oxidant to generate benzoquinone electrophiles from hydroquinone proelectrophiles. Phenolic compounds, such as hydroquinone and catechol, have been reported to be oxidized by metal oxides and enzymes present in soil samples (42, 43), including manganese(III,IV) (hydr)oxides (44–46). Manganese(III,IV) (hydr)oxides are especially important oxidants in soils and at the oxic/anoxic interface in sediments. Near the oxic/anoxic interface manganese cycles through the +II and +III/+IV oxidation states via abiotic and microbial processes, causing hundreds of redox reactions before ultimate burial (47, 48). Manganese(II) is highly soluble and diffuses to the oxic zone where it is oxidized by microorganisms or O$_2$ to manganese(III,IV) solids that settle back to the oxic/anoxic zone (47–53). Although Mn$^{III,IV}$ (hydr)oxides are thought to be ubiquitous in soils and sediments, they are less abundant in nature than
Fe$^{III}$ oxides (54). Despite being less abundant, Mn$^{III,IV}$ (hydr)oxides have higher reduction potentials than Fe$^{III}$ oxides (54–57), so they are likely to represent the active oxidant for proelectrophiles such as hydroquinone moieties within NOM.

Synthesized MnO$_2$(pyrolusite) particles are available to us that contain essentially all Mn$^{IV}$ and no Mn$^{III}$ (58–61); reducing this solid will generate one Mn$^{II}$(aq) for each Mn$^{IV}$ which requires two electrons to be transferred:

$$\text{Mn}^{IV}O_2 + 2e^- + 4H^+ \leftrightarrow \text{Mn}^{II}(aq) + 2H_2O \quad (3.2)$$

Henceforth, we will refer to MnO$_2$(s, pyrolusite) as MnO$_2$ for simplicity. We expect MnO$_2$ will oxidize hydroquinone XH$_2$ proelectrophiles at pH <12 based on thermodynamic considerations. An $E_h$–pH diagram (Figure S3.2 in the Supporting Information) shows graphically that MnO$_2$ can oxidize three model hydroquinone proelectrophiles at pH <12 but the reduction potential of goethite (Fe$^{III}$OOH(s) is too low to oxidize hydroquinone proelectrophiles above pH 3.5.

Sulfur, nitrogen, and oxygen atoms are the most common nucleophilic sites within organic compounds. Lone pair electrons residing on these atoms are donated during reaction mechanisms including Michael addition (62, 63). Nucleophilic addition reactions with quinones in conditions representative of aquatic environments have been reported for hydrogen sulfide (64), sulfite (65), and anilines (4, 66–69). Also, nucleophilic addition reactions between glutathione and $p$-benzoquinone have been studied under physiologic conditions (27).

Many nucleophilic organic compounds are oxidizable by MnO$_2$, including phenolic compounds (46) and anilines (70). The mechanism shown in Figure 3.1 and Figure 3.2 does not account for oxidative consumption of the nucleophile or reactions of the oxidation
products of the nucleophile. We avoid this complication by carefully selecting model nucleophiles that are not appreciably oxidized under our experimental conditions.

In this study, we conduct kinetic experiments using 4-ethylaniline (An) and 4-methylimidazole (Im) as convenient added nucleophiles. These model nucleophiles are not significantly oxidized under our experimental conditions, they are amenable to analysis by HPLC, and they contain only one potentially nucleophilic atom. Using these model nucleophiles will allow us to compare adduct formation and multiple generations for two different N-donor nucleophiles. An possesses a pKₐ of 5.00 (71) and serves as a model aromatic amine.

\[
\ce{\overset{\text{NH}}{\text{NH}}N \rightleftharpoons \overset{\text{NH}}{\text{NH}} + H^+} \tag{3.3}
\]

Im serves as a model for histidine and other heterocyclic nitrogen compounds. Im has a pKₐ of 7.54 (71):

\[
\ce{\overset{\text{HN}}{\text{HN}} \rightleftharpoons \overset{\text{HN}}{\text{HN}} + H^+} \tag{3.4}
\]

In Chapter 2, An was shown to be a stronger nucleophile than Im in terms of observed reaction rate with p-benzoquinone. We saw that the rate constant corrected for the deprotonated species \(k_{\text{Nu}^-}\) was 2.5 times larger for An than for Im. In this chapter, our experiments will also be conducted at pH 7.0 and we expect that An will act as a stronger nucleophile than Im.

Our kinetic studies will focus on the effect of increasing the concentration of proelectrophile or nucleophile. Increasing the concentration of proelectrophile will promote the rate of benzoquinone generation, while increasing the concentration of nucleophile will increase the rate of adduct formation. We can indirectly observe multiple generations by monitoring consumption of proelectrophile \(\text{(XH}_2\text{)}\) and nucleophile \(\text{(Nu)}\), and generation of \(\text{Mn}^{\text{II}}\text{(aq)}\). \(\text{XH}_2\) is only consumed when it oxidized to the first generation \(\text{Z}\), generating
Additional generation of Mn$^{\text{II}}$ indicates oxidation of one of the adducts. Nu is only consumed by addition to a benzoquinone. Greater consumption of Nu than XH$_2$ indicates the formation of diadducts or triadducts containing Nu (e.g. X–(Nu)$_2$). Ratios of Nu consumption and Mn$^{\text{II}}$ generation to XH$_2$ consumption over reaction time graphically illustrate this effect.

It should be noted that there are a number of possible side reactions that could occur other than those shown on Figure 3.1 or Figure 3.2. It is likely that not all of the intermediates and products will be stable in solution, especially with an oxidant present: they and may also undergo other decomposition reactions such as ring-opening.

Compounds containing aniline as a functional group are capable of forming imine products via a rapid and reversible reaction 1,2-nucleophilic addition reaction (66, 72):

\[
\text{NH}_2 + \text{R} \quad \Rightarrow \quad \text{R} \text{NH} \text{O} + \text{H}_2\text{O}
\]

Whether an imine or a Michael type product is favored depends on the electrophilicity of the benzoquinone substrate. In some cases both products are formed: for 4-methylaniline and 2,6-dimethyl-$p$-benzoquinone a 3:1 product ratio of Michael addition product to imine was reported (72), while a 3:2 product ratio was reported for benzidine (73). We expect that EWG substituted model proelectrophiles will favor Michael-type additions. Any imine products that do form will most likely revert to reactants (66) ultimately leading to Michael-type
adducts. In aqueous solutions, the solvent H₂O drives the equilibrium for imine formation (as written in Reaction 3.5) towards the reactants, but H₂O is not involved in the equilibrium for Michael addition.

Radical coupling reactions could also take place in our proelectrophile experiments. Proelectrophile compounds used in this study are phenols (Table 3.1); phenolic compounds are known to undergo oxidative coupling reactions that leads a to a complex array of products (74). Anilines can also form radical-coupling products following 1 e⁻ oxidation (70). Whether nucleophilic addition or radical-coupling reactions dominate in our reactions is difficult to discern. Most literature reports support nucleophilic addition because the reported products are consistent with products of a Michael addition pathway, but they have been unable to definitively prove that Michael addition occurs and radical coupling does not (5, 16, 67, 68, 75). We sought to minimize cross-coupling reactions in our experiments by using low concentrations of proelectrophiles and oxidant, anticipating that only extremely low concentration of radicals will be generated. Colarieti et al. (42, 43) observed polymerization caused by oxidants in soil samples when 0.5 g/L (5 mM) of phenol or 5.26 mM and catechol was added to soil samples containing approximately 0.2 g/L (2.3 mM) loading of manganese oxides. In our experiments, the proelectrophile concentration was only 100 µM and never exceeded 1 mM and the loading of MnO₂ was 200 µM. Unfortunately, we are limited in our ability to conclude that only nucleophilic addition occurs and radical addition does not; rather, they more likely occur simultaneously.

In this study we demonstrate that simple experiments consisting of model substituted hydroquinone proelectrophiles and nucleophiles provide evidence of the complex proelectrophile pathway including the generation of multiple addition products and that generating highly reactive electrophiles from electron-withdrawing group bearing proelectrophiles leads to fast rates of nucleophile consumption, despite competition with H₂O and OH⁻.
First, we chose a proelectrophile-nucleophile pair to study stoichiometry and kinetics of consumption of the proelectrophile and nucleophile in the presence of MnO$_2$. Second, we compare four proelectrophiles and two nucleophiles to explore effects of molecular structure on consumption rates and generation of Mn$^{II}$(aq). Last, we will employ LC/MS to acquire mass spectral evidence of monoadduct formation for nine different nucleophiles with an EWG substituted hydroquinone proelectrophile. By using simple model compounds that represent nucleophilic contaminants and potential binding sites in NOM, we hope to show that electrophiles generated in-situ by oxidation in real soils and sediments may represent an important sink for nucleophilic contaminants.

3.2 Materials and Methods

All aqueous solutions were prepared from reagent grade chemicals and distilled, deionized water (Milli-Q water, 18 MΩ-cm resistivity, Millipore Corp., Milford, MA). All bottles and glassware were rinsed with distilled water, soaked in 5 M nitric acid overnight, rinsed with distilled water and Milli-Q water and then air-dried.

3.2.1 Chemicals

3-(N-Morpholino)propanesulfonic acid (MOPS) and ascorbic acid were purchased from Sigma (St. Louis, MO). 2,5-Dihydroxyterephthalic acid (XH$_2$(IV)), 4-ethylaniline, 4-methylimidazole, 4-aminophenol, L-glutathione, 2-aminobenzyl alcohol, 4-chloroaniline, and 4-nitrophenol were purchased from Aldrich (St. Louis, MO). 4-Nitroaniline was purchased from Alfa Aesar (Pelham, NH). 2′,5′-Dihydroxyacetophenone (XH$_2$(II)), 4-hydroxyphenylacetone and methyl 4-hydroxybenzoate were purchased from TCI America (Portland, OR). NaCl was purchased from Acros Organics (Fair Lawn, NJ). Manganese
atomic absorption standard solution and HPLC and LC/MS Optima grade methanol were purchased from Fisher Scientific (Pittsburgh, PA). 2,5-Dihydroxybenzoic acid (XH$_2$(III)), hydroquinone (XH$_2$(I)), L-cysteine, 2-mercaptoethanol, and ammonium acetate were purchased from Fluka (Buchs, Switzerland). NaOH and acetic acid were purchased from J. T. Baker (Phillipsburg, NJ).

Previously synthesized MnO$_2$ particles were available for the present work (58–61). D-spacings determined using selected area electron diffraction coincided with values reported in the literature for the mineral pyrolusite. The average oxidation state of Mn in the particles was determined to be +3.98 by iodometric titration: the Mn in the particles is 98% Mn$^{IV}$ and 2% Mn$^{III}$ (60).

### 3.2.2 Experimental Design

Experiments were conducted in 100 mL polypropylene bottles placed in a constant temperature water bath held at 25 ± 1°C and continuously stirred with Teflon coated stir bars. Sufficient stock solution of MOPS buffer, NaCl, proelectrophile, and nucleophile were added to achieve a total volume of 100 mL and 5 mM MOPS, 10 mM NaCl concentration. Initial concentration of proelectrophile and nucleophile varied between 0 and 1000 µM. To minimize dissolved oxygen, solutions were continuously sparged with N$_2$(g). HCl and NaOH were added to adjust the pH to 7.0. Solutions were allowed to equilibrate for 30 min. Reaction was initiated by the pipetting a small volume (<1 mL) of well mixed 0.22 M MnO$_2$ stock slurry that was available in our laboratory. In tests lacking MnO$_2$ only negligible decrease of any proelectrophile or nucleophile concentration was observed within the time scale of our experiments. Following addition of MnO$_2$, 5 mL samples were periodically withdrawn by syringe and filtered through 0.2 µm track-etch polycarbonate filter membrane
syringe filters (Whatman, UK). Filtration quenches any reaction caused by MnO$_2$ particles. No other attempt was made to quench reactions in the samples. Filter quenched samples gave the same HPLC, LC/MS and AAS results when analyzed immediately and after 2 days of storage.

Control experiments lacking a proelectrophile were performed for ten nucleophiles. The initial concentration of nucleophile was 100 µM and MnO$_2$ was spiked to 200 µM concentration in 5 mM MOPS buffer adjusted to pH 7.0 and 10 mM NaCl. After 1 hr, samples were filtered and analyzed for dissolved Mn using flame atomic absorption spectrometry and nucleophile concentration using LC/MS. No significant concentration of dissolved Mn was detected, and no significant change in nucleophile concentration was observed.

3.2.3 Instrumentation and Analysis

Our experiments were performed in the absence of chelating agents, hence dissolved concentrations of Mn$^{III}$ and Mn$^{IV}$ can be assumed to be negligible. Dissolved Mn, measured using flame atomic absorption spectrophotometry (FAAS, AA Analyst 100, Perkin-Elmer, Norwalk, CT), is assumed to consist entirely of Mn$^{II}$. Any Mn$^{2+}$ adsorped to the MnO$_2$ particles removed via filtration would not have been detected. The limit of detection (LOD) for FAAS was calculated using the regression residual standard deviation ($S_{y/x}$)

$$LOD = 3.3 \frac{S_{y/x}}{m}$$

(3.6)

where $m$ is the slope of the calibration curve (76–78).

4-Ethylaniline, 4-methylimidazole and acetylhydroquinone (XH$_2$(II)) concentrations were monitored by an HPLC system (Waters, Milford, MA) using a UV absorbance detection at 230 nm. Run conditions were isocratic using 70% 5 mM ammonium acetate and 30%
methanol as the eluent at a flow rate of 1 mL min\(^{-1}\). Separation was achieved using a \(\mu\)Bondapak 5\(\mu\)m 3.6 \(\times\) 300 mm column (Waters, Milford, MA). Acetyl-\(p\)-benzoquinone, Z(II), was not detected using this method.

LC/MS with electrospray ionization was used to detect addition products in experiments where 2,5-dihydroxybenzoic acid (XH\(_2\)) served as the proelectrophile. LC separation was conducted using a Waters 2795 separation module (Milford, MA) with a 4.6 \(\times\) 100 mm, 5 \(\mu\)m particle size, Atlantis T3 column (Waters, Milford, MA) run in isocratic mode with 70\% 5 mM ammonium acetate and 30\% methanol eluent at a flow rate of 200 \(\mu\)L min\(^{-1}\). Adducts were identified based on their molecular ion ([M-H]\(^-\)) and any detected fragments, typically [M-COOH]\(^-\).

Initial rates were calculated for experiments with acetylhydroquinone, 4-ethylaniline, and MnO\(_2\). Initial rates were determined by applying linear regression to XH\(_2\)(II), 4-ethylaniline, and Mn\(^{II}\)(aq) versus time data for the first 4 data points. All data points used in regression were taken within 10 minutes of the start of the reaction. The slope obtained by regression corresponds to initial rate of consumption for nucleophile or proelectrophile or generation of Mn\(^{II}\) (\(r_0\) in units of \(\mu\)M min\(^{-1}\)). Linear regression analysis yielded correlation coefficients (\(r^2\)) that were greater than 0.8, with the exception of one experiment with 500 \(\mu\)M initial concentration of 4-ethylaniline. The time course data and best-fit lines are shown in the Supporting Information (Figure S3.4 and Figure S3.5).
3.3 Results and Discussion

3.3.1 Proelectrophile Experiments with Acetylhydroquinone, 4-Ethylaniline, and MnO₂

We chose test nucleophiles that were not significantly oxidized by MnO₂ under the conditions and timescales of our experiments. We chose nucleophiles that yielded Mn^{II}(aq) below the detection limit (3 µM) after one hour of contact time with MnO₂. Our final set of nucleophiles includes substituted phenols, anilines, and 4-methylimidazole (Table S3.1). Nucleophiles containing thiol groups are quickly oxidized by MnO₂ and were not used in any further experiments.

In one series of kinetic and stoichiometric experiments acetylhydroquinone (XH₂(II), Table 3.1) served as the test proelectrophile and 4-ethylaniline (An) served as the test nucleophile. Oxidation of aniline and related compounds by Mn^{III,IV} (hydr)oxide minerals, such as MnO₂, has been reported (70). Under the conditions and timescale of our experiments, no Mn^{II} was detected in slurries containing An and MnO₂ (Table S3.1). We chose XH₂(II) as the proelectrophile because the acetyl group (–C(=O)CH₃) has similar electron-withdrawing properties as a carboxyl group, which are believed to be highly prevalent in NOM. The Hammett constant, σᵢ, is a way to quantify electron withdrawing properties of a substituent group. For a carboxylic acid (–C(=O)OH) σᵢ is close to σᵢ for the acetyl group: +0.45 and +0.5, respectively (79). Also, the carboxyl group in XH₂(III) imparts a negative charge at pH 7, but XH₂(II) is neutral at that pH avoiding electrostatic affects related to charge. In experimental solutions lacking MnO₂ no change in XH₂(II) or An concentration was observed within 2 hours. Also, no change in XH₂(II) concentration was observed in buffered
solutions lacking MnO$_2$ and An. Similarly, no change in An concentration was observed in buffered solutions lacking MnO$_2$ and XH$_2$(II).

An example time course is shown in Figure 3.3A where the initial concentrations were 225 µM XH$_2$(II), 100 µM An, and the MnO$_2$ loading was 200 µM in 5 mM MOPS buffer (pH 7.0) and 10 mM NaCl. Under these conditions the Mn$^{II}$(aq) concentration exceeded 130 µM after 90 min. XH$_2$(II) and An were not completely consumed, their concentrations approached 30 µM and 15 µM, respectively.

The ratios of Mn$^{II}$ generated to XH$_2$(II) consumed and An consumed to XH$_2$(II) consumed give insight into what reactions occur following the oxidation of XH$_2$(II) to the benzoquinone (Z) form, on a per XH$_2$(II) oxidized basis (Figure 3.3B). The ratio of Mn$^{II}$(aq) generated to XH$_2$(II) consumed (R$_A$) reflects the number of oxidation steps the proelectrophile undergoes, on average.

$$R_A = \frac{\text{Mn}^{II}(aq) \text{ generated}}{\text{XH}_2(\text{II}) \text{ consumed}} = \frac{\text{Mn}^{II}(aq)}{[\text{XH}_2(\text{II})]_0 - [\text{XH}_2(\text{II})]}$$ (3.7)

This ratio would be equal to 1 if the proelectrophile was only oxidized once because of the one-to-one stoichiometry of MnO$_2$ oxidation of hydroquinones. R$_A$ greater than 1 imply that adducts are becoming oxidized, consuming more MnO$_2$ and generating more Mn$^{II}$. Note that R$_A$ would be confounded by adsorption of Mn$^{II}$: the observed values would be too low. The ratio of An consumed to XH$_2$(II) consumed (R$_B$) gives the average number of An molecules that reacted with the Z form of XH$_2$(II), because An is only consumed by forming adducts with Z.

$$R_B = \frac{\text{An consumed}}{\text{XH}_2(\text{II}) \text{ consumed}} = \frac{[\text{An}]_0 - [\text{An}]}{[\text{XH}_2(\text{II})]_0 - [\text{XH}_2(\text{II})]}$$ (3.8)
If $R_B$ is equal to 1, then one $Z$ reacted with one $An$, presumably leading to a monoadduct. If $R_B$ is less than 1, we expect that some of $Z$ reacted with $H_2O$ or $OH^-$ to form hydration products. $R_B$ greater than 1 suggests that diadducts were formed. Note that if the oxidized form or $Z$ persists in solution the ratio would be lower than expected.

In Figure 3.3B, $R_A$ approaches 1.4, while $R_B$ approaches 0.5. Both ratios are approximately 0.75 at the onset of reaction and stop changing significantly after 30 min.

The ratios shown in Figure 3.3B change depending on the concentration of $An$ and $XH_2(II)$ (Figure 3.4). $R_B$ increases with increasing concentration of $An$, but was relatively constant with time. In Figure 3.4A, the concentration of $An$ is increased from 50 to 1000 µM, the concentration of $XH_2(II)$ was 100 µM, and the MnO$_2$ loading was 200 µM. When $An$ was 50 µM (half the concentration of $XH_2(II)$) the ratio was close to 0.5. The remaining 50 µM of $Z(II)$ that is generated most likely reacts with $OH^-$ or $H_2O$ to form hydration products. When $An$ was 100 µM, $R_B$ starts at 1, but decreases over time. As $An$ is consumed hydration becomes more favorable and $R_B$ decreases. At higher $An$ concentrations $R_B$ was consistently higher than 1. High concentrations of $An$ will favor formation of the adducts over hydration products and should promote the formation of diadducts.

$R_A$ exceeded 1 for all concentrations of $An$ (Figure 3.4B). The ratio of Mn$^{II}$ generated to $XH_2$ consumed reflects the number of generations of benzoquinones ($Z$) that are formed via oxidation. Using our Mn$^{IV}$O$_2$ pyrolusite, $R_A$ would not exceed 1.0 if only a first generation formed. Even in the absence of $An$, $R_A$ was higher than 1 after 10 min, suggesting that the $Z$ form reacts with $H_2O$ or $OH^-$ forming the hydration product (hydroxylated acetylhydroquinone). The hydration product is oxidized leading to a second generation benzoquinone, a second Mn$^{II}$, and a larger Mn$^{II}$ to $XH_2$ ratio. When $An$ is present, $R_A$ increases with increasing concentration of $An$. In other words, more Mn$^{II}$ is generated when 4-ethylaniline forms an addition product with acetyl benzoquinone than when only hydration
occurs. An did not reduce MnO$_2$ in experiments lacking XH$_2$, but oxidation of An in these experimental slurries would increase the observed concentration of Mn$^{II}$(aq) and increase $R_A$.

$R_A$ represents oxidation steps and $R_B$ represents An addition steps that occur following oxidation of XH$_2$(II) by MnO$_2$ (on average). Increasing [Nu]$_0$ increases both ratios, evidence that the formation of second-generation benzoquinones and diadducts is promoted (Figure 3.4). Forming an adduct with An could promote subsequent oxidation and addition reactions because the redox properties of the monoadduct (X-Nu) differ from the parent XH$_2$(II) and likely also differ from the hydration product (X-OH). In the monoadduct, An is a substituent of the aromatic ring (replacing a hydrogen in XH$_2$(II)). In the hydration product, a hydrogen in XH$_2$(II) has been replaced with a hydroxyl group. An and hydroxyl groups should both be electron-donating groups (EDG) which would give X-Nu a lower reduction potential than XH$_2$(II), making it more easily oxidized. Recall that Hammett constants are a way to quantify electronic influence of substituents. For a hydroxyl group the Hammett substituent constant, $\sigma_p$, is $-0.37$ (80). Although we could not locate a $\sigma_p$ that would represent An as a substituent, the $\sigma_p$ for the somewhat similar $-\text{NHCH}_3$ group is $-0.7$ (80). A $-\text{NHCH}_3$ substituted addition product should be approximately twice as easily oxidized as the $-\text{OH}$ substituted hydration product assuming that the Hammett relationship is linear for this reaction. The second-generation addition products (diadducts) have another An or $-\text{OH}$ substituent furthering lowering their reduction potential. When the concentration of An is increased the formation of X-Nu will be favored over X-OH, favoring more the more easily oxidizable products which could lead to further oxidation by MnO$_2$.

Initial rates for consumption of An and XH$_2$(II) and generation of Mn$^{II}$(aq) increased when the concentration of XH$_2$(II) was increased from 0 to 1000 $\mu$M and the concentration of An was 100 $\mu$M (Figure 3.5A). The rate of generation of Mn$^{II}$ is similar to the rate
of consumption of \( \text{XH}_2\text{(II)} \) at \([\text{XH}_2\text{(II)}]_0\) lower than 250 \( \mu \text{M} \). At \([\text{XH}_2\text{(II)}]_0\) greater than 500 \( \mu \text{M} \), generation of \( \text{Mn}^{\text{II}} \) is faster than consumption of \( \text{XH}_2\text{(II)} \).

At low \([\text{XH}_2\text{(II)}]_0\) \( \text{Mn}^{\text{II}} \) might be generated only from oxidation of \( \text{XH}_2\text{(II)} \). At higher \([\text{XH}_2\text{(II)}]_0\), oxidation of adducts would lead to more \( \text{Mn}^{\text{II}} \) being generated than \( \text{XH}_2 \) being consumed.

The consumption rate of \( \text{An} \) is tied to the rate of \( \text{XH}_2 \) oxidation because the reaction of \( \text{An} \) with \( \text{Z} \) can only occur after oxidation. However, the rate consumption of \( \text{An} \) is consistently slower than the rate of consumption of \( \text{XH}_2\text{(II)} \) and generation of \( \text{Mn}^{\text{II}} \) (Figure 3.5A). \( \text{An} \) competes with \( \text{H}_2\text{O} \) or \( \text{OH}^- \) to react with available \( \text{Z} \). The concentration of \( \text{An} \) is 100 \( \mu \text{M} \) in Figure 3.5A which will also limit the increase in rate of consumption of \( \text{An} \).

When the concentration of \( \text{An} \) was increased from 0 to 1000 \( \mu \text{M} \) and the concentration of \( \text{XH}_2\text{(II)} \) was 100 \( \mu \text{M} \), the initial rate of consumption of \( \text{XH}_2\text{(II)} \) and generation of \( \text{Mn}^{\text{II}} \) did not depend on the concentration of \( \text{An} \), but the initial rate of consumption of \( \text{An} \) did increase nonlinearly (Figure 3.5B).

There may be several explanations for the limited increase in rate of consumption of \( \text{An} \) with increasing \([\text{An}]_0\). Consumption of \( \text{An} \) could be limited by available surface sites; however, the rate of consumption of \( \text{XH}_2\text{(II)} \) should decrease with increasing \([\text{An}]_0\) as surface sites become occupied by \( \text{An} \) unless adsorption of \( \text{An} \) does not affect adsorption of \( \text{XH}_2\text{(II)} \). The generation of \( \text{Mn}^{\text{II}} \) and consumption of \( \text{XH}_2\text{(II)} \) did not depend on \([\text{An}]_0\), suggesting that the amount of \( \text{Z} \) available may have limited the rate of consumption of \( \text{An} \).

At low \([\text{An}]_0\), \( \text{An} \) competes with \( \text{H}_2\text{O}/\text{OH}^- \) to react with \( \text{Z} \) and the rate of consumption of \( \text{An} \) increases with \([\text{An}]_0\). At high \([\text{An}]_0\) all \( \text{Z} \) the rate of consumption of \( \text{An} \) is limited by the rate of generation of \( \text{Z} \).
3.3.2 Influence of Proelectrophile and Nucleophile Structure

Time course data for proelectrophile experiments employing 160 µM 4-ethylaniline (An) or 4-methylimidazole (Im) as the nucleophile are shown in Figure 3.6. Reaction slurries contained 100 µM of a single proelectrophile (shown in Table 3.1) and 200 µM MnO$_2$ in 10 mM NaCl and 5 mM MOPS adjusted to pH 7. After 90 min of contact time, total An consumed depended on proelectrophile identity: XH$_2$(III) >XH$_2$(I) >XH$_2$(II) >XH$_2$(IV) (Figure 3.6A). When Im was the nucleophile the order was XH$_2$(III) >XH$_2$(II) >XH$_2$(IV) >XH$_2$(I) (Figure 3.6B). Regardless of proelectrophile identity consumption of An was greater than Im after 90 min. Note that no reaction was observed between any of the proelectrophiles and either nucleophile in the absence of MnO$_2$.

Protonation state could explain the greater consumption of An than Im (Figure 3.6): the pK$_a$ of Im is 7.54 and the pK$_a$ of An is 5.00 (71). Thus, at pH 7.0, 77% of Im is protonated while only 1% of An is protonated. Protonation blocks nucleophilic attack by occupying the lone-pair electrons on atom of the nucleophile that would donate those electrons during nucleophilic attack.

For both An and Im, we observed the greatest consumption of nucleophile when XH$_2$(III) was the proelectrophile (Figure 3.6A and B) and the least with XH$_2$(I). Z(I) lacks an electron-withdrawing substituent and should be the least electrophilic among the four benzoquinones. The electron-withdrawing carboxylic acid substituent on Z(III) leads to the greater consumption of nucleophile.

More An was consumed when the proelectrophile was XH$_2$(III) than when it was XH$_2$(II). As mentioned previously, the Hammett constant, $\sigma_p$, is $+0.45$ for a carboxylic acid (–C(=O)OH), and $+0.5$ for a acetyl group (–C(=O)CH$_3$); acetyl groups should be slightly more electron withdrawing than carboxylic acids (79). However, at pH 7 carboxylic
acid groups would be deprotonated and $\sigma_p$ is only +0.11 for carboxylate ($\text{C(=O)}\text{O}^-$). At pH 7, Z(III) would be significantly less electrophilic than Z(II). Figure 3.6C shows less Mn$^{\text{II}}$(aq) was generated with XH$_2$(II) than XH$_2$(III): less Z(II) was generated than Z(III). The consumption of nucleophile will depend on both the concentration of Z and the rate constant for nucleophilic attack. In this case, even though we expect Z(II) to be more electrophilic than Z(III), less generation of Z(II) from XH$_2$(II) ultimately lead to less consumption of An.

Similarly, less Mn$^{\text{II}}$ was generated when XH$_2$(IV) was the proelectrophile than for XH$_2$(II) (Figure 3.6C), but more An was consumed after 90 min (Figure 3.6A). Electronic effects of proelectrophile substituents influence both the rate of oxidation of the XH$_2$ form and the rate of nucleophilic addition to the Z form. Both rates will influence the extent of nucleophile consumption: XH$_2$(IV) is more difficult to oxidize than XH$_2$(II), but the corresponding Z form should be a stronger electrophile.

With XH$_2$(I), the least An and Im was consumed, and Mn$^{\text{II}}$ increased quickly but plateaued near 100 $\mu$M within 15 min (Figure 3.6C and D). If all 100 $\mu$M of XH$_2$(I) were consumed, 100 $\mu$M Mn$^{\text{II}}$ would be generated if only the parent proelectrophile was oxidized and not for any addition or hydration products. Mn$^{\text{II}}$ approached 100 $\mu$M for both An and Im when XH$_2$(I) or XH$_2$(IV) served as the proelectrophile. With XH$_2$(III) and An Mn$^{\text{II}}$ exceeded 100 $\mu$M, but not with XH$_2$(III) and Im. The 100 $\mu$M limit was exceeded for both nucleophiles when XH$_2$(II) was the proelectrophile.

In the case of An and XH$_2$(I), 50 $\mu$M of An is consumed, presumably forming addition products with the generated $p$-benzoquinone (Figure 3.6A). However, the addition and hydration products have not been further oxidized, which would have lead to Mn$^{\text{II}}$ concentration in excess of the 100 $\mu$M limit. For XH$_2$(IV), the 100 $\mu$M Mn$^{\text{II}}$ limit was not exceeded within 90 min for either nucleophile (Figure 3.6C and D). Because it contains two electron-
withdrawing carboxylic acid functional groups, 2,5-dihydroxyterephthalic acid, $\text{XH}_2(\text{IV})$, should be the most difficult of these proelectrophiles to oxidize; accordingly, 160 μM of An or Im had no appreciable affect on oxidation rate. For $\text{XH}_2(\text{II})$, the 100 μM Mn$^{\text{II}}$ limit was exceeded within 30 min. Similar results for $\text{XH}_2(\text{II})$ are obtained in the absence of an added nucleophile. Oxidation of $\text{XH}_2(\text{II})$ in the presence of Im quickly approached the limit, but did not exceed it. This was also observed in the absence of nucleophile. In contrast, the 100 μM Mn$^{\text{II}}$ limit was quickly exceeded when An was present. It is difficult to tell why the presence of Im limited the amount of Mn$^{\text{II}}$ that was generated (Figure 3.6D) but An did not (Figure 3.6C). Perhaps the lower reduction potential, and thus increased oxidizability, of an An adduct over $\text{XH}_2(\text{III})$ lead to further reduction of MnO$_2$. Im did not form adducts as well as An, so there were no adducts to reduce any available MnO$_2$.

It is important to note that under conditions where MnO$_2$ is not completely reduced to Mn$^{\text{II}}$ the fraction that is adsorbed to MnO$_2$ particles would not be detected. However, our results still show that hydroquinone proelectrophiles act as more than two equivalent reductant via multiple generations of adducts. Oxidation of hydroquinone proelectrophiles to benzoquinone electrophiles requires two electron transfer. Reduction of MnO$_2$ to Mn$^{\text{II}}$ also requires two electron transfer because our MnO$_2$ particles contain almost entirely Mn$^{\text{IV}}$. The balanced redox reaction is:

$$\text{MnO}_2(s) + \text{XH}_2(aq) + 2\text{H}^+ \rightleftharpoons \text{Mn}^{\text{II}}(aq) + Z(aq) + 2\text{H}_2\text{O} \quad (3.9)$$

Thus if $\text{XH}_2$ could only be a two-equivalent reductant, the maximum amount of Mn$^{\text{II}}$ that could be generated would be equal to the initial concentration of $\text{XH}_2$ or loading of MnO$_2$, whichever is smaller. However, this threshold is exceeded in many of our experimental results (for example, Figure 3.6). If the measured concentrations of Mn$^{\text{II}}$ are too small
because of adsorption the number of generations observed would increase, but our conclusion that multiple generations occur is unchanged.

### 3.3.3 LC/MS Evidence of Monoadducts

We obtained mass spectral evidence for nine monoadducts using LC/MS with XH₂(III) as the proelectrophile. Figure 3.7 shows two example spectra taken after 12 hours of contact time from separate reaction slurries with 50 µM XH₂(III) as the proelectrophile, 100 µM 4-hydroxybenzoate as the nucleophile, 220 µM MnO₂ in 10 mM NaCl and 5 mM MOPS buffer adjusted to pH 7.0. Negative mode electrospray LC/MS requires analytes to have an ionizable functional group that becomes negatively charged during the electrospray process (87). Because XH₂(III) has a carboxylic acid group, the products X-Nu and X-OH are amenable to LC/MS detection regardless of the identity of the nucleophile. Figure 3.7A shows the total ion count chromatogram for an experiment where 4-hydroxybenzoate was the nucleophile. Peaks are observed for XH₂(III), X-OH, X-Nu, and 4-hydroxybenzoate. The phenolic hydroxy group in methyl 4-hydroxybenzoate is sufficiently ionizable to allow LC/MS detection. The monoadduct could be one of three isomers depending on which ring hydrogen is replaced by the nucleophile. Unfortunately, it is not possible to discern which monoadduct isomer is formed to because mass spectrometry typically cannot discern isomers, especially when peak detection is limited to molecular ion and a single fragment. Electrospray typically does not generate many fragments. Unfortunately, authentic standards are not available for these compounds, and quantification of adducts was not possible.

Negative mode electrospray ionization mass spectrometry gives a signal corresponding to the molecular weight of anionic fragments. For monoadducts of methyl 4-hydroxybenzoate the deprotonated anion, [M-H]−, and an anion resulting from loss of a carboxyl group,
[M-COOH]$^-$, were detected (Figure 3.7). (M represents the mass of the total molecular structure.) For methyl 4-hydroxybenzoate, a third anion resulting from loss of a methoxy group, [M-OCH$_3$]$^-$, was also observed (Figure 3.7A). Note that mass spectra are reported as mass to charge ratio (m/z), under our LC/MS conditions the charge is always $-1$, so the m/z is equal to the molecular weight of the fragment.

Nucleophile and monoadduct structures are shown in Table 3.2, along with m/z of identifying fragments. [M-H]$^-$ and [M-COOH]$^-$ fragments were detected for all observed adducts, except when para-aminophenol was the nucleophile. Mass spectra are shown in the supporting information (Figure S3.7). Only one of the three possible isomers is shown in Table 3.2. A peak with an m/z of 169, corresponding to the hydration product, was also observed (Figure 3.7A). We did not obtain any mass spectral evidence for diadducts.

Prior researchers have focused on anilines for their environmental significance (4, 13, 14, 24, 67, 82). Also, we observed monoadducts for four para-substituted anilines with our model proelectrophile XH$_2$(III), supporting the conclusion that anilines could be removed by addition reactions to electrophilic sites in natural organic matter (NOM) generated by oxidation (Table 3.2). We observed a monoadduct with 4-methylimidazole, a N-heterocycle. Previously, it was reported that the N-heterocycle pyridine did not bind irreversibly to sediment samples (13). Our data suggests that perhaps irreversible binding might have been observed if an oxidant had been added to (or present in) the soil samples.

Despite the slow rate of addition of phenols to p-benzoquinone at pH 7.0 (Table 2.1), we detected monoadducts of three phenolic nucleophiles with XH$_2$(III) as the proelectrophile (Table 3.2). Nucleophilic reactions of phenolic compounds have received less attention in the literature than anilines. This observation could have implications for the environmental behavior of phenolic compounds, given the ubiquity of both manganese (hydr)oxides in soils and sediments and phenolic functional groups in NOM, natural compounds and synthetic
compounds. Perhaps this is another mechanism that could explain phenolic coupling reactions. Phenols (42) and catechols (43) oxidized by metal oxides and enzymes present in soil samples polymerize when the phenol or catechol is in excess. This is usually attributed to fast radical coupling reactions, but a proelectrophile pathway could also be active. Whether the radical coupling or addition pathway dominates will depend on the competitive reactions of the phenoxy radical formed by one electron oxidation. Because radicals are so reactive, the unpaired electron will be either quickly donated to another phenol via a cross-coupling reaction or lost to oxidation. In the case of hydroquinone, catechol and other 1,2- or 1,4-dihydroxybenzenes, losing the second electron to oxidation generates an electrophilic benzoquinone. Once the electrophilic form is generated it can react with phenolic nucleophiles leading to Michael addition products. Ultimately, reaction conditions control the dominant pathway: excess phenol or catechol favors donating the unpaired electron in radical reactions, while excess oxidant favors losing the electron via oxidation.

3.4 Conclusions

We explored the proelectrophile pathway by employing simple substituted hydroquinone proelectrophiles and nucleophiles as model compounds using MnO$_2$ as an oxidant. We used 4-ethylaniline (An) and 4-methylimidazole (Im) as model nucleophiles because they are not oxidized significantly by MnO$_2$ under the conditions of our experiments and represent some key nitrogen-containing functional groups in organic contaminants and biologically relevant compounds. Experiments investigating reaction stoichiometry of proelectrophile experiments with An and acetylhydroquinone (XH$_2$(II)) show that increasing An increases the fraction of Z(II) that undergoes nucleophilic addition of An instead of hydration and

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increase the likelihood that di or triadducts are generated. Lastly, using LC/MS we were able to acquire mass spectral evidence of monoadducts formed between MnO$_2$ oxidized XH$_2$(III) and nine nucleophiles, including ones we previously found to react very slowly with $p$-benzoquinone.

Hydroquinone functional groups within NOM could be capable of acting as proelectrophiles when an oxidant is available, such as Mn$^{III,IV}$ (hydr)oxides. These groups would be particularly electrophilic when bonded to electron-withdrawing carboxylic acid functional groups. The prevalence of hydration versus nucleophile addition product as well as the formation of multiple addition products depends on reaction conditions. Higher concentrations of added nucleophiles favors Michael addition over hydration and promotes the generation of multiple addition products.

Acknowledgments

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Table 3.1. Hydroquinone proelectrophiles (XH\textsubscript{2}) discussed in this chapter and available chemical properties. \(E_{H^\circ}^o\) is the standard reduction potential at 25°C for the reaction \(Z + 2H^+ + 2e^- \rightleftharpoons XH_2\). \(pK_{a1}\) and \(pK_{a2}\) correspond to the phenolic hydrogens. \(pK_{aR}\) refers to the side group of the reduced form (XH\textsubscript{2}). \(pK_a\) values reported for zero ionic strength and 25°C.

<table>
<thead>
<tr>
<th>(XH_2)</th>
<th>(Z)</th>
<th>(pK_{a1})</th>
<th>(pK_{a2})</th>
<th>(pK_{aR})</th>
<th>(E_{H^\circ}^o) (V)</th>
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<td>hydroquinone</td>
<td></td>
<td>10.16\textsuperscript{a}</td>
<td>12.02\textsuperscript{a}</td>
<td>N/A</td>
<td>0.699\textsuperscript{a}</td>
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<tr>
<td>I</td>
<td>(\text{O} \quad \text{O})</td>
<td>(\text{O} \quad \text{O})</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>acetylhydroquinone</td>
<td></td>
<td>9.48\textsuperscript{b}</td>
<td>12.90\textsuperscript{b}</td>
<td>N/A</td>
<td>(e)</td>
</tr>
<tr>
<td>II</td>
<td>(\text{O} \quad \text{O} \quad \text{CH}_3 \quad \text{O} \quad \text{O} \quad \text{CH}_3)</td>
<td>(\text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O})</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2,5-dihydroxybenzoic acid</td>
<td></td>
<td>10.47\textsuperscript{c}</td>
<td>(e)</td>
<td>2.96\textsuperscript{c}</td>
<td>0.769\textsuperscript{d}</td>
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<tr>
<td>III</td>
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<td>(\text{O} \quad \text{O} \quad \text{O} \quad \text{O})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,5-dihydroxyterephthalic acid</td>
<td></td>
<td>(e)</td>
<td>(e)</td>
<td>(e)</td>
<td>(e)</td>
</tr>
<tr>
<td>IV</td>
<td>(\text{O} \quad \text{O} \quad \text{OH} \quad \text{OH})</td>
<td>(\text{O} \quad \text{O} \quad \text{O} \quad \text{O})</td>
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</tr>
</tbody>
</table>

\textsuperscript{a}Ref. 35. \textsuperscript{b}Ref. 83. \textsuperscript{c}Ref. 71 \textsuperscript{d}Ref. 34. \textsuperscript{e}Data not available.
Table 3.2. Addition products for nine nucleophiles and MnO$_2$ oxidized XH$_2$(III) detected by negative-mode electrospray LC/MS after 12 hours of contact time. Only one of three possible isomers is shown. “n.d.” indicates nucleophiles that are not detectable by electrospray LC/MS in negative mode. The molecular weight (MW) in g mol$^{-1}$, molecular ion mass to charge ratio (m/z) and major fragment m/z are shown for each structure.

<table>
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<th>Nucleophile</th>
<th>MW</th>
<th>Adduct</th>
<th>MW</th>
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<td><img src="image1" alt="Image" /></td>
<td>275</td>
</tr>
<tr>
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<td>[M-H]$^{-}$ = 122</td>
<td><img src="image2" alt="Image" /></td>
<td>[M-H]$^{-}$ = 274</td>
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<tr>
<td>4-ethylaniline</td>
<td>121</td>
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</tr>
<tr>
<td></td>
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<td>[M-H]$^{-}$ = 272</td>
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<td>4-chloroaniline</td>
<td>127</td>
<td><img src="image5" alt="Image" /></td>
<td>279</td>
</tr>
<tr>
<td></td>
<td>n.d.</td>
<td><img src="image6" alt="Image" /></td>
<td>[M-H]$^{-}$ = 278</td>
</tr>
<tr>
<td>4-aminophenol</td>
<td>109</td>
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<td>261</td>
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<td>[M-H]$^{-}$ = 108</td>
<td><img src="image8" alt="Image" /></td>
<td>[M-H]$^{-}$ = 260</td>
</tr>
<tr>
<td>4-nitrophenol</td>
<td>139</td>
<td><img src="image9" alt="Image" /></td>
<td>291</td>
</tr>
<tr>
<td></td>
<td>n.d.</td>
<td><img src="image10" alt="Image" /></td>
<td>[M-H]$^{-}$ = 290</td>
</tr>
<tr>
<td>4-hydroxyphenyl acetone</td>
<td>150</td>
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<td>302</td>
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<tr>
<td></td>
<td>[M-H]$^{-}$ = 149</td>
<td><img src="image12" alt="Image" /></td>
<td>[M-H]$^{-}$ = 301</td>
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<tr>
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<td>136</td>
<td><img src="image13" alt="Image" /></td>
<td>288</td>
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<tr>
<td></td>
<td>[M-H]$^{-}$ = 135</td>
<td><img src="image14" alt="Image" /></td>
<td>[M-H]$^{-}$ = 287</td>
</tr>
<tr>
<td>4-methylbenzoate</td>
<td>152</td>
<td><img src="image15" alt="Image" /></td>
<td>304</td>
</tr>
<tr>
<td></td>
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<td><img src="image16" alt="Image" /></td>
<td>[M-H]$^{-}$ = 303</td>
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<tr>
<td>4-methylimidazole</td>
<td>82</td>
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<td>234</td>
</tr>
<tr>
<td></td>
<td>n.d.</td>
<td><img src="image18" alt="Image" /></td>
<td>[M-H]$^{-}$ = 233</td>
</tr>
</tbody>
</table>

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Figure 3.1. Starting with a di-substituted hydroquinone, oxidation followed by Michael addition can occur sequentially. Abbreviations are provided below each intermediate product denoting hydroquinone (X) and benzoquinone (Z) structures.
Figure 3.2. Starting with a monosubstituted hydroquinone, oxidation followed by Michael addition can occur sequentially. Abbreviations are provided below each intermediate and product denoting hydroquinone (X) and benzoquinone (Z) structures. All possible products are shown in Figure S3.3.
Figure 3.3. (A) Example time course for experimental slurry containing 4-ethylaniline (An) and acetylhydroquinone (XH$_2$(II)) in the presence of MnO$_2$. Initial conditions: 50 µM An, 100 µM XH$_2$(II), 200 µM MnO$_2$ in 5 mM MOPS buffer adjusted to pH 7.0 and 10 mM NaCl. (B) Mn$^{II}$ to XH$_2$(II) and An to XH$_2$(II) ratio.
Figure 3.4. (A) $R_A$, Mn$^{II}$(aq) generated to acetylhydroquinone (XH$_2$(II)) consumed. (B) $R_B$, 4-ethylaniline (An) consumed to acetylhydroquinone (XH$_2$) consumed. Initial conditions: 100 µM XH$_2$ and 200 µM MnO$_2$ in 5 mM MOPS buffer adjusted to pH 7.0 and 10 mM NaCl. Dashed lines mark one-to-one ratio.
Figure 3.5. Initial rate ($r_0$) of consumption of $XH_2(II)$, consumption of 4-ethylaniline (An) and generation of $Mn^{II}(aq)$. For simplicity, both generation and consumption are shown as positive. (A) 100 µM An. (B) 100 µM $XH_2(II)$. Conditions: 5 mM MOPS adjusted to pH 7.0, 10 mM NaCl, 200 µM MnO$_2$. 
Figure 3.6. Nucleophile consumption and Mn$^{II}$(aq) generation for 4-ethylaniline (An, A and C) and 4-methylimidazole (Im, B and D) in the presence of one of four proelectrophiles (Table 3.1). Conditions: 100 µM proelectrophile, 160 µM nucleophile, and 200 µM MnO$_2$ in 5 mM MOPS adjusted to pH 7 and 10 mM NaCl.
Figure 3.7. Example chromatogram (A) and mass spectrum (B) collected by LC/MS after 12 hours of contact time from a slurry containing 50 µM XH₂(III), 220 µM MnO₂, and 100 µM methyl 4-hydroxybenzoate. The mass spectrum in B corresponds to the peak in A with retention time of 12.8 min. Conditions: 5.0 mM MOPS adjusted to pH 7.0, 10 mM NaCl.
3.5 Supporting Information

Supporting information for Chapter 3 includes a section discussing an application of the Langmuir-Hinshelwood-Hougan-Watson kinetic model, an $E_{\text{h}}$-pH diagram depicting the dependence of redox potential for reduction of MnO$_2$ and goethite and oxidation of three substituted hydroquinones (Figure S3.2) as well as tabulated equilibrium constants used to generate Figure S3.2 (Table S3.3, Table S3.4, and Table S3.5), tabulated 1 hour Mn$^{II}$ concentration measured in slurries of candidate nucleophiles in the absence of an XH$_2$ proelectrophile (Table S3.1), structures of all possible hydroquinone and benzoquinone adducts that can be generated from proelectrophile experiments with an XH$_2$ proelectrophile containing a single substituent group (Figure S3.3), a graphical determination of the initial rates shown in Figure 3.5 (Figure S3.4 and Figure S3.5), additional time course data where the MnO$_2$ loading was 80 or 160 µM (Figure S3.6), mass spectra supporting Table 3.2 (Figure S3.7), and a table of available acid dissociation constants ($pK_a$) for chemical compounds studied in this chapter (Table S3.2).

3.6 Application of the Langmuir-Hinshelwood-Hougan-Watson kinetic model

The Langmuir-Hinshelwood-Hougan-Watson (LHHW) kinetic model, which was developed for surface-catalyzed reactions, can be applied to redox reactions at mineral surfaces (1–3). The LHHW model considers a reaction at a surface that involves three steps: adsorption to an available surface site, reaction at the surface (in this case electron transfer), and desorption of the product. We can apply the LHHW model to oxidation of XH$_2$(II) at the
surface of MnO$_2$ and using initial rate data to discern the rate-determining step from one of those three processes (2). The relationship between initial rate of XH$_2$(II) consumption and initial XH$_2$(II) concentration is mathematically different depending on which process is rate determining. In the adsorption-limited case, sorption of XH$_2$(II) to the surface determines the rate of consumption of XH$_2$(II). The initial rate ($r_0^a$) is linearly dependent on initial concentration:

$$r_0^a = -\frac{d[XH_2(II)]}{dt} = k_{ads}[S][XH_2(II)]_0 \quad (S3.1)$$

where $k_{ads}$ is the rate constant for adsorption, and S represents surface sites. If oxidation of XH$_2$(II) at the surface is rate-determining step then the initial rate depends on the amount of XH$_2$(II) that is adsorbed to the surface. In this case, adsorption will reach equilibrium conditions because XH$_2$(II) can adsorb to the surface faster than it can react at the surface.

$$r_0^s = -\frac{d[XH_2(II)]}{dt} = \frac{k_{ox}[S][XH_2(II)]_0}{1 + K_{XH_2(II)}[XH_2(II)]_0} \quad (S3.2)$$

where $k_{ox}$ is the rate constant for oxidation at the surface of MnO$_2$. Adsorption of XH$_2$(II) is represented by the equilibrium constant ($K_{XH_2(II)}$) because the amount of XH$_2$(II) that adsorbs is not kinetically controlled. In the third case, where consumption of XH$_2$(II) is limited by desorption of Z(II) and not by adsorption of XH$_2$(II) or oxidation at the surface, the rate of desorption is independent of the concentration of XH$_2$(II). Under initial conditions, the concentration of Z(II) is zero, so the rate expression becomes:

$$r_0^d = -\frac{d[XH_2(II)]}{dt} = k_{des}[S] \quad (S3.3)$$

Where $k_{des}$ is the rate constant for desorption of the oxidation product. Equations S3.1, S3.2, and S3.3 predict different dependence of initial rate of consumption of XH$_2$(II) on the initial
concentration of XH$_2$(II). Depending on which equation best describes the data, the LHHW model gives some evidence of whether adsorption, reaction at the surface, or desorption of the product is rate determining for consumption of the substrate. Note that Equation S3.2 can mimic Equation S3.1 under conditions where the product of $K_{XH_2(II)}[XH_2(II)]_0$ is small.

The above equations assume that electron transfer at the surface is so fast that XH$_2$ is oxidized to Z(II) and MnO$_2$ is reduced to Mn$^{II}$ in a single step. In other words, the first electron transfer is not modeled separately from the second electron transfer step. This assumption describes a situation where the semiquinone radical intermediate and with Mn$^{III}$ form Z(II) and Mn$^{II}$ faster than any other potential reactions.

In Figure S3.1, the nonlinear increase in initial rate of consumption of XH$_2$(II) with increasing XH$_2$(II) concentration is consistent with the surface-reaction limited case (Equation S3.2, shown as a line Figure S3.1A). Values for $k_{ox}[S]$ and $K_{XH_2(II)}$ are determined by nonlinear least squares regression. Individual values for $k_{ox}$ and [S] cannot be determined using this method, but the aggregate parameter $k_{ox}[S]$ was determined to be $3.4 \times 10^{-7}$ M s$^{-1}$. $K_{XH_2(II)}$ was $4 \times 10^6$ M$^{-1}$. This suggests that oxidation of XH$_2$(II) at the surface of MnO$_2$ controls the overall rate of reaction. Increasing the concentration of XH$_2$(II) in the bulk solution increases the rate of reaction until the concentration is high enough to saturate the surface.

Figure S3.1A and Equation S3.2 concern the oxidation of XH$_2$(II) on the surface of MnO$_2$. What is not addressed is whether the adduct-forming reaction of Z(II) with An occurs on the surface or if Z(II) must desorb to react with An in the bulk phase. A relationship similar to Equation S3.2 can written for consumption of An. Consider the case where An must adsorb to the surface of MnO$_2$ prior to reaction with adsorbed Z(II). The initial rate of consumption of An would be described by an equation similar to Equation S3.2 for the
surface reaction between An and Z(II).

\[
r_0 = -\frac{d[An]}{dt} = \frac{k[Z(II)][S][An]_0}{1 + K_{An}[An]_0}
\]  

(S3.4)

where \( K_{An} \) is the adsorption equilibrium constant. Alternatively, consider the case where Z(II) must desorb to react with An in the bulk phase. The rate of consumption of An should increase linearly with increasing concentration of An:

\[
r_0 = -\frac{d[An]}{dt} = k[An][Z(II)]
\]  

(S3.5)

Note that we expect that Z(II) will not accumulate, so \([Z(II)]\) should be small and independent of \([An]_0\).

The rate of consumption of An shown in Figure S3.1B is not consistent with Equation S3.5; it appears to follow Equation S3.4, such that the amount of An available to react is limited by the adsorption coefficient and available adsorption sites. Similar to Equation S3.2, the parameters in Equation S3.4 can be determined from least squares regression. In this case, the aggregate parameter is \( k[Z(II)][S] \) and its value was \( 2 \times 10^{-7} \text{ M s}^{-1} \). \( K_{An} \) was \( 6 \times 10^6 \text{ M}^{-1} \). The rate of consumption of An cannot continually increase with increasing \([An]_0\) because the number of surface sites is limited. Because Z(II) is generated on the solid surface, it might not persist long enough to desorb to the bulk phase. It would react with a nearby adsorbed nucleophile or \( \text{H}_2\text{O}/\text{OH}^- \). Unfortunately, it is not possible to discern if the increase in An consumption in Figure S3.1B is due to this surface effect, or if increasing the concentration of An increases the rate of nucleophilic addition of An over hydration. Most likely, time course data for the products (X-Nu and X-OH) would be required to separate these two effects.
It is important to note that the LHHW model was developed for simple irreversible reactions at surfaces that act as catalysts and are not consumed during the reaction (1). MnO$_2$ is subject to reduced to Mn$^{II}$ and is dissolved during our experiments. When XH$_2$(II) is present in excess of MnO$_2$ the solid particles become completely dissolved (Figure S3.5) and heterogeneous kinetics no longer apply. However, we applied the LHHW model only to initial rates. Under initial conditions there is still MnO$_2$ surface present regardless of the concentration of XH$_2$(II). Also, the LHHW model only provides meaningful relationships between the initial rate of XH$_2$(II) and [XH$_2$(II)]$_0$ and between the initial rate of An consumption and [An]$_0$ because it was developed to model A $\rightarrow$ B reactions at catalyst surfaces. For this reason only equations S3.2 and S3.4 are plotted on Figure S3.1.

The results of our LHHW modeling exercise imply a reaction mechanism where both the rate of consumption of XH$_2$(II) and An are limited by the rate of reaction at the MnO$_2$ surface. Before any reaction occurs, XH$_2$(II) and An sorb and accumulate on the surface of MnO$_2$. The first electron transfer takes place where XH$_2$(II) is oxidized to the semiquinone radical and an Mn$^{IV}$ atom at the surface is reduced to Mn$^{III}$. Both the semiquinone and Mn$^{III}$ are sufficiently reactive that they are quickly transformed to Z(II) and Mn$^{II}$, respectively. The highly electrophilic Z(II) reacts quickly with either An, OH$^-$, or H$_2$O to form the monoadduct (X-An) or the hydration product (X-OH). At some unknown later time, Mn$^{II}$ and X-An or X-OH desorb from the MnO$_2$ surface.
Table S3.1. Mn^{II}(aq) after 1 hour in filtered samples of candidate nucleophiles and MnO$_2$, which serves as a measure of the extent of reaction. ND indicates that Mn^{II}(aq) was below the 3 µM limit of detection. Conditions: 100 µM candidate nucleophile, 200 µM MnO$_2$, 5 mM MOPS buffer (pH 7.0) and 10 mM NaCl. Acid dissociation values pK$_a$ are shown for 25°C and zero ionic strength when available (4).

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>pK$_a$</th>
<th>Mn^{II}(aq) (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl 4-hydroxy-benzoate</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>4-Hydroxy-phenylacetone</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>4'-Hydroxy-acetophenone</td>
<td>8.05</td>
<td>ND</td>
</tr>
<tr>
<td>4-Nitrophenol</td>
<td>7.15</td>
<td>ND</td>
</tr>
<tr>
<td>4-Ethylaniline</td>
<td>5.00</td>
<td>ND</td>
</tr>
<tr>
<td>4-Chloro-aniline</td>
<td>4.06</td>
<td>ND</td>
</tr>
<tr>
<td>4-Methyl-imidazole</td>
<td>7.54</td>
<td>ND</td>
</tr>
<tr>
<td>No added nucleophile</td>
<td></td>
<td>ND</td>
</tr>
</tbody>
</table>
Table S3.2. Selected acid dissociation constants reported at 25 °C. Values adjusted to zero ionic strength using the Davies equation when necessary (5).

<table>
<thead>
<tr>
<th>Use</th>
<th>Chemical</th>
<th>log K</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleophile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydroxylamine</td>
<td>5.96</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.74</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4-Ethylaniline</td>
<td>5.00</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4-Methoxyaniline</td>
<td>5.357</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><em>p</em>-Aminophenol&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.64</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>4-Chloroaniline</td>
<td>4.07</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4′-Aminoacetophenone</td>
<td>2.29</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Aniline</td>
<td>4.601</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4-Nitroaniline</td>
<td>1.015</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4-Methylimidazole</td>
<td>7.54</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2-Mercaptoethanol&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.72</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4-Nitrophenol</td>
<td>7.15</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4′-Hydroxyacetophenone</td>
<td>8.05</td>
<td>4</td>
</tr>
<tr>
<td><strong>Proelectrophile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydroquinone</td>
<td>10.16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.02</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Gentisic acid&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.96</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.47</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Acetylhydroquinone</td>
<td>9.48</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.9</td>
<td>7</td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td>4.784</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>MES</td>
<td>6.270</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>MOPS</td>
<td>7.184</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>CHES</td>
<td>9.384</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data only available for one of two pK<sub>a</sub> values. <sup>b</sup> Data only available for two of three pK<sub>a</sub> values.
Table S3.3. Reduction potential thermodynamic data used to generate Figure S3.2. Values reported for 25°C No activity corrections were made.

<table>
<thead>
<tr>
<th>Reduction potential</th>
<th>$E^\circ_H$ (V)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Z-R} + 2 e^- + 2 H^+ \rightleftharpoons H_2X - R$</td>
<td>0.769</td>
<td>9</td>
</tr>
<tr>
<td>$\text{Fe}^{3+} + e^- \rightleftharpoons \text{Fe}^{2+}$</td>
<td>0.777</td>
<td>5</td>
</tr>
<tr>
<td>$\text{MnO}_2$ (pyrolusite) $+ 2 e^- + 4 H^+ \rightleftharpoons \text{Mn}^{2+} + 2 \text{H}_2\text{O}$</td>
<td>1.229</td>
<td>11</td>
</tr>
<tr>
<td>$\text{O}_2 + 4 e^- + 4 H^+ \rightleftharpoons 2 \text{H}_2\text{O}$</td>
<td>1.23$^a$</td>
<td>5</td>
</tr>
<tr>
<td>$2 H^+ + 2 e^- \rightleftharpoons \text{H}_2$</td>
<td>0.00</td>
<td>5</td>
</tr>
</tbody>
</table>

$^a$ Calculated from reported $p\epsilon^\circ$: $E_0 = \frac{2.303RT}{F} p\epsilon^\circ$. 

$E^\circ_H = 2.303RT \ln \frac{p\epsilon^\circ}{F}$. 

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Table S3.4. Equilibrium thermodynamic data used to generate Figure S3.2. Acid dissociation constants adjusted for zero ionic strength using the Davies equation when necessary (5). Values reported at 25°C.

<table>
<thead>
<tr>
<th>Equilibrium expression</th>
<th>log K</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( XH_2 = ) <img src="image" alt="Structure" /> ( R = \text{COOH} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( H_3X \rightleftharpoons H_2X^- + H^+ )</td>
<td>2.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>( H_2X^- \rightleftharpoons HX^{2-} + H^+ )</td>
<td>10.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13</td>
</tr>
<tr>
<td>( HX^{2-} \rightleftharpoons X^{3-} + H^+ )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( R = \text{H} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( H_2X \rightleftharpoons HX^- + H^+ )</td>
<td>10.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10</td>
</tr>
<tr>
<td>( HX^- \rightleftharpoons X^{2-} + H^+ )</td>
<td>12.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10</td>
</tr>
<tr>
<td>( R = \text{CH}_3 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( H_2X \rightleftharpoons HX^- + H^+ )</td>
<td>10.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td>( HX^- \rightleftharpoons X^{2-} + H^+ )</td>
<td>12.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Acid dissociation constant for carboxylic acid side group.  
<sup>b</sup> Acid dissociation for first phenolic proton (−OH).  
<sup>c</sup> Data not available.  
<sup>d</sup> Acid dissociation constant for second phenolic proton.
Table S3.5. Stability constants\(^a\) for Fe\(^{II}\) and Fe\(^{III}\) hydrolysis used to generate Figure S3.2 (14).

<table>
<thead>
<tr>
<th>Equilibrium Expression</th>
<th>logK</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Fe}^{II}\text{OH}^+ \rightleftharpoons \text{Fe}^{2+} + \text{OH}^-)</td>
<td>9.397</td>
<td>4</td>
</tr>
<tr>
<td>(\text{Fe}^{II}(\text{OH})_2^0 \rightleftharpoons \text{Fe}^{2+} + 2 \text{OH}^-)</td>
<td>20.494</td>
<td>4</td>
</tr>
<tr>
<td>(\text{Fe}^{II}(\text{OH})_3 \rightleftharpoons \text{Fe}^{2+} + 3 \text{OH}^-)</td>
<td>28.494</td>
<td>4</td>
</tr>
<tr>
<td>(\text{Fe}^{II}(\text{OH})_4^{2-} \rightleftharpoons \text{Fe}^{2+} + 4 \text{OH}^-)</td>
<td>45.988</td>
<td>4</td>
</tr>
<tr>
<td>(\text{Fe}^{III}\text{OH}^{2+} \rightleftharpoons \text{Fe}^{3+} + \text{OH}^-)</td>
<td>2.187</td>
<td>4</td>
</tr>
<tr>
<td>(\text{Fe}^{III}(\text{OH})_2^+ \rightleftharpoons \text{Fe}^{3+} + 2 \text{OH}^-)</td>
<td>4.594</td>
<td>4</td>
</tr>
<tr>
<td>(\text{Fe}^{III}(\text{OH})_3^0 \rightleftharpoons \text{Fe}^{3+} + 3 \text{OH}^-)</td>
<td>13.600</td>
<td>4</td>
</tr>
<tr>
<td>(\text{Fe}^{III}(\text{OH})_4^{-} \rightleftharpoons \text{Fe}^{3+} + 4 \text{OH}^-)</td>
<td>21.588</td>
<td>4</td>
</tr>
<tr>
<td>(\text{Fe}^{II}_2(\text{OH})_2^{4+} \rightleftharpoons 2 \text{Fe}^{3+} + 2 \text{OH}^-)</td>
<td>2.854</td>
<td>4</td>
</tr>
<tr>
<td>(\text{Fe}^{III}_3(\text{OH})_3^{5+} \rightleftharpoons 3 \text{Fe}^{3+} + 4 \text{OH}^-)</td>
<td>6.288</td>
<td>4</td>
</tr>
<tr>
<td>(\text{Mn}^{II}(\text{OH})^+ \rightleftharpoons \text{Mn}^{2+} + \text{OH}^-)</td>
<td>10.0</td>
<td>4</td>
</tr>
<tr>
<td>(\text{Mn}^{II}(\text{OH})_3^{-} \rightleftharpoons \text{Mn}^{2+} + 3 \text{OH}^-)</td>
<td>34.2</td>
<td>4</td>
</tr>
</tbody>
</table>

*Solubility product constants*

<table>
<thead>
<tr>
<th>Expression</th>
<th>logK</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Fe}^{II}\text{OOH(s,geothite)} + \text{H}_2\text{O} \rightleftharpoons \text{Fe}^{3+} + 3 \text{OH}^-)</td>
<td>0.200</td>
<td>5</td>
</tr>
<tr>
<td>(\text{Mn}^{II}(\text{OH})_2(s) \rightleftharpoons \text{Mn}^{2+} + \text{OH}^-)</td>
<td>14.50</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^a\) 25°C. Adjusted for zero ionic strength using the Davies equation (5).
Figure S3.1. (A) Initial rate of consumption of XH$_2$(II), $r_0$(XH$_2$). (B) Initial rate of consumption of 4-ethylaniline, $r_0$(An). For simplicity, rates of consumption are shown as positive. (A) 100 µM An, line represents Equation S3.2. (B) 100 µM XH$_2$(II), line represents Equation S3.4. Conditions: 5 mM MOPS adjusted to pH 7.0, 10 mM NaCl, 200 µM MnO$_2$. 

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Figure S3.2. $E_h$-pH diagram for three benzoquinone/hydroquinone half-reactions including MnO$_2$ / Mn$^{2+}$(aq), FeOOH(s, goethite), and O$_2$ / H$_2$O half-reactions as reference. Conditions: 50 µM of each hydroquinone and benzoquinone, 50 µM Mn$^{2+}$(aq) and total Fe$^{II}$, the activity of MnO$_2$ or FeOOH(s, goethite) is set to 1.0, P$_{O_2}$ = 0.21 atm, 10 mM NaCl and 25°C temperature.
Figure S3.3. Molecular structures of all possible adducts and benzoquinone electrophiles that could result from a hydroquinone electrophile with a single substituent (R).
Figure S3.4. Proelectrophile experiments consisting of 4-ethylaniline (An) as the nucleophile and acetylhydroquinone (XH$_2$(II)) as the proelectrophile. Lines represent linear fits used to determine initial rate. Conditions: 100 µM XH$_2$(II), 200 µM Mn$^{II}$(aq), 5 mM MOPS adjusted to pH 7.0, and 10 mM NaCl.
Figure S3.5. Proelectrophile experiments consisting of 4-ethylaniline (An) as the nucleophile and acetylhydroquinone (XH₂(II)) as the proelectrophile. Lines represent linear fits used to determine initial rate. Conditions: 100 µM An, 200 µM MnO₂, 5 mM MOPS adjusted to pH 7.0, and 10 mM NaCl.
Figure S3.6. Proelectrophile experiments consisting of 4-ethylaniline (An) as the nucleophile and acetylhydroquinone (XH₂(II)) as the proelectrophile. Conditions: 130 µM XH₂(II), 5 mM MOPS adjusted to pH 7.0, and 10 mM NaCl.
Figure S3.7. Mass spectra corresponding to monoadducts in Table 3.2 collected after 12 hrs. Conditions: 50 µM gentisic acid proelectrophile, 100 µM nucleophile, 220 µM MnO$_2$, 5 mM MOPS (pH 7.0), and 10 mM NaCl.
Figure S3.8. Continued.
(a) 4-hydroxyacetophenone

(b) methyl 4-hydroxybenzoate

(c) 4-methylimidazole

**Figure S3.9.** Continued.
References


(3) W. A. Arnold, A. L. Roberts. Pathways and kinetics of chlorinated ethylene and chlorinated acetylene reaction with Fe(0) particles. *Environmental Science and Technology* 2000, 34, 1794–1805.


Chapter 4

Permanganate Oxidation of Alkenes: Studying Product Distribution Using *cis*-Stilbenedicarboxylic Acid as a Convenient Model Compound

4.1 Introduction

Permanganate (MnO$_4^-$) is used in many ways in water treatment and environmental remediation (1). It is employed in a third of water treatment facilities in the United States. MnO$_4^-$ can remove Mn$^{II}$ and Fe$^{II}$ from drinking water (2) as well as remedy taste and odor problems (3). MnO$_4^-$ can be used to help remove arsenic from drinking water by oxidizing arsenite (As$^{III}$) to arsenate (As$^{V}$) which is more easily removed by precipitation or adsorption (4). It can remove precursors to trihalomethanes and other disinfection byproducts (5). It promotes coagulation and flocculation in waters with high organic content improving removal of organic carbon (6, 7). MnO$_4^-$ in-situ chemical oxidation can effectively treat many contaminants in the subsurface including chlorinated ethylenes (8–10), cyclotrimethylenetrinitramine (RDX, 11, 12), chlorophenols (13), and methyl-tert-butyl ether (14). Researchers are discovering that many pollutants that are effectively removed
by MnO$_4^-$ including the cyanobacterial toxin microcystin-LR (15–18), estradiol related endocrine-disrupting compounds (19), the antibacterial triclosan (20), carbamazepine (21), ciprofloxacin, lincomycin, and trime-thoprim (22, 23), and pesticides including aldrin, terbufos, metribuzin, permethrin (24), and dichlorvos (25).

In all of these situations, it is important to recognize (1) the impacts of solution conditions on the efficiency and efficacy of oxidation by MnO$_4^-$ and (2) what the ultimate products are following treatment. Alkenes (−C=C−) are a simple MnO$_4^-$ oxidizable functional group that occurs in many organic chemicals. We will employ cis-stilbenedicarboxylic acid (SDCA) as a model alkene to examine alkene oxidation by MnO$_4^-$. Using SDCA allows us to probe how pH and reactant concentrations affect kinetics and influence the ultimate product distribution in the laboratory instead of complicated environmental samples.

Alkenes oxidation by MnO$_4^-$ is believed to occur via cyclic Mn$^V$ intermediate that subsequently breaks down via multiple steps into the ultimate products (26). Rate expressions are reported to be first-order in both MnO$_4^-$ and in alkene, have small positive enthalpies of activation, and large negative entropies of activation (27–30) implying an intermediate formed by attack of one MnO$_4^-$ onto one alkene:

\[
\begin{array}{c}
\text{CH}_2\text{CH} + \text{Mn}^{VII}O \rightarrow \text{CH}_2\text{Mn}^{V}O
\end{array}
\] (4.1)

Solution pH within the range of aquatic environments can have a complex effect on alkene oxidation by MnO$_4^-$. For some compounds, a higher protonation state leads to an increase in reaction rate in the pH range 0.5-5. For example, for substituted maleic acids
the oxidation rate constant of the monoanion (HA–) was faster than for the neutral (H₂A) or dianion (A²⁻) species (31–33), which was attributed to hydrogen bonding in the monoanion leading to a more rigid planar structure (33). Conversely, for fumaric acids the order was 
H₂A > HA– > A²⁻ which was attributed to electrostatic effects (34). For trans-crotonic acid the neutral species reacts faster than the anion (35). However, no effect of pH on oxidation rate was observed for trichloroethylene (10) or carbamazepine (21) in environmentally relevant pH ranges.

The final product distribution depends on solution conditions, in particular pH and the ratio of alkene substrate and MnO₄⁻ concentrations will determine the dominant reaction pathway. Which products are ultimately generated depends on what reactions occur following the generation of the cyclic Mn⁵ intermediate. The intermediate can undergo further oxidation by MnO₄⁻, intramolecular reactions, acid-base chemistry, or hydrolysis reactions.

An example of further oxidation by MnO₄⁻ is the formation of a cyclic Mn⁶ intermediate (30, 36, 37):

\[
\begin{align*}
\text{H} & \text{C} \text{O} \quad \text{Mn}^\text{V} \quad \text{O} \\
\text{H} & \text{C} \text{O} \quad \text{Mn}^\text{V} \quad \text{O} \\
\end{align*}
\xrightarrow{\text{MnO}_4^-}
\begin{align*}
\text{H} & \text{C} \text{O} \quad \text{Mn}^\text{VI} \quad \text{O} \\
\text{H} & \text{C} \text{O} \quad \text{Mn}^\text{VI} \quad \text{O} \\
\end{align*}
\]

A Mn⁶ intermediate reportedly formed during oxidation of cinnamic acid at acidic pH (38) and for bicyclo[2.2.1]-2-heptene (39). Lee and Brownridge (38) reasoned that because manganate (Mn⁶O₄²⁻) is known to cleave diols, any cleavage of the diol-like cyclic Mn⁶ intermediate would not occur until after Mn⁵ is oxidized to Mn⁶. To illustrate this point,
they added periodate ($\text{IO}_4^-$), which oxidizes any $\text{Mn}^\text{V}$ to $\text{Mn}^\text{VI}$, increasing the yield of cleavage products.

We expect that excess $\text{MnO}_4^-$ will favor oxidation by a second $\text{MnO}_4^-$. Wiberg and Saegebarth (40) reported UV spectral evidence for a $\text{Mn}^\text{VI}$ intermediate with excess $\text{MnO}_4^-$. However, in the case of oleate and crotonate, a cyclic $\text{Mn}^\text{VI}$ intermediate was reported when the concentration of the substrate was an order of magnitude greater than $\text{MnO}_4^- (30)$.

A ketol product can be generated from either the $\text{Mn}^\text{V}$ or $\text{Mn}^\text{VI}$ intermediate (shown for an $\text{Mn}^\text{VI}$ intermediate in Reaction 4.3). First, hydrolysis of the intermediate leads to an acyclic Mn ester. In Reaction 4.3, two-electron reduction of $\text{Mn}^\text{VI}$ within the ester yields an $\text{Mn}^\text{IV}$ product and the ketol (39, 41).

\[
\begin{align*}
\text{H}_2\text{C}=\text{O} &= \text{Mn}^\text{VI} & \text{H}_2\text{C}=\text{O} &= \text{Mn}^\text{VI} = \text{O} \\
\text{H}_2\text{C}=\text{O} &= \text{Mn}^\text{VI} = \text{O} \\
\text{H}_2\text{C}=\text{O} &= \text{Mn}^\text{VI} = \text{O} \\
\end{align*}
\]

(4.3)

Overall, 4 electrons are transferred from the alkene substrate to $\text{MnO}_4^-$. Ketol products were favored at neutral pH for oleic acid (39), maleic acid and fumaric acid (42), and methylated uracil derivatives (41). The ketol product is not expected to form via oxidation of a diol, as was reported for oleate (43), uracil (41) and propylene glycol (44).

Excess $\text{MnO}_4^-$ leads to a dione product. One proposed mechanism (38, 45) involves oxidation of a tertiary hydrogen in the intermediate to a carbocation which is further oxidized
to the dione (Reaction 4.4).

\[
\begin{array}{c}
\text{H-C-O} \\
\text{H-C-O} \\
\text{Mn}^{\text{VI}}
\end{array}
\xrightarrow{\text{Mn}^{\text{VII}O^-}}
\begin{array}{c}
\text{C} \\
\text{H} \\
\text{O} \\
\text{Mn}^{\text{VI}}
\end{array}
\xrightarrow{\text{O}}
\begin{array}{c}
\text{C} \\
\text{O}
\end{array}
\] (4.4)

Note that MnO$_4^-$ has been reported to oxidize tertiary hydrogens (46). Another possibility is that the dione can result from oxidation of the ketol (Reaction 4.5).

\[
\begin{array}{c}
\text{C} \\
\text{O}
\end{array}
\xrightarrow{\text{H-C-OH}}
\begin{array}{c}
\text{Mn}^{\text{VII}O^-}
\end{array}
\xrightarrow{\text{+}}
\begin{array}{c}
\text{C} \\
\text{O}
\end{array}
\xrightarrow{\text{+ HMn}^{\text{V}O_4^2-}}
\] (4.5)

The dione product results from an overall 6 electron transfer from the alkene to MnO$_4^-$. Breakdown of either the Mn$^\text{V}$ or the Mn$^\text{VI}$ cyclic intermediate can lead to two aldehyde products by cleaving the C−C bond (26). Two aldehyde products result from an overall 4 electron transfer from alkene substrate to MnO$_4$.

\[
\begin{array}{c}
\text{H-C-O} \\
\text{H-C-O} \\
\text{Mn}^{\text{V}}
\end{array}
\xrightarrow{\text{+ Mn}^{\text{VII}O^-}}
\begin{array}{c}
\text{H-C-O} \\
\text{H-C-O} \\
\text{Mn}^{\text{III}OH^2}
\end{array}
\] (4.6)

Breakdown of the Mn$^\text{V}$ intermediate at acidic pH yielding aldehyde products was reported for acetylene carboxylic acid (47), crotonic acid (35), maleic acid and fumaric acid (34,
48), and cinnamic acid (38). Under highly acidic conditions, protonation of the cyclic hypomanganate ester accelerates decomposition (similar to Equation 4.6) by increasing the oxidation potential of Mn$^V$ (45). UV/Vis spectra of the inorganic products match Rayleigh’s law indicating that the inorganic product is particulate or colloidal Mn$^{IV}$O$_2$ (27, 29). Mn$^{III}$ does not disproportionate when chelated by pyrophosphate and is the ultimate MnO$_4^-$ reduction product when pyrophosphate is present (34, 35, 38, 49, 50). However, in the absence of a chelating ligand disproportionation of Mn$^{III}$ yields Mn$^{IV}$O$_2$ particles and Mn$^{2+}$(aq) (37).

Carboxylic acids are often reported as the final products of MnO$_4^-$ oxidation of alkenes (26). MnO$_4^-$ is known to oxidize aldehydes to carboxylic acids via a pathway where loss of the aldehydic hydrogen is thought to represent the rate-determining step (51–55). This reaction can be acid catalyzed

$$\begin{array}{c}
\text{Oxidation of an alkene resulting in C=C bond cleavage and two carboxylic acid final products is an overall 8 electron transfer to MnO}_4^- \text{ from the alkene substrate.}
\end{array}$$
A diol product can be formed from hydrolysis of either the Mn$^V$ or Mn$^{VI}$ cyclic ester intermediate (Equation 4.9). Diol products have been reported from MnO$_4^-$ oxidation of cycloalkanes (27, 56–58), uracils (41), bicyclo[2.2.1]-2-heptene (39), stilbene (58), and oleate (43, 59). At alkaline pH, the high concentration of OH$^-$ favors hydrolysis of the cyclic Mn$^V$ intermediate. The diol product forms by an overall 2 electron transfer from the alkene to MnO$_4^-$. 

To summarize, consider that the stoichiometry of MnO$_4^-$ and alkene substrate depends on the number of electrons transferred to form the ultimate reaction products. The possible alkane oxidation products we are considering are two carboxylic acids (resulting from cleavage of the C=C bond), one carboxylic acid and one aldehyde, two aldehydes, dione, ketol, and diol. The MnO$_4^-$ reduction products we will consider are MnO$_2$(s), MnOOH(s) and Mn$^{2+}$(aq). Half-reactions show the number of electrons required to oxidize and alkene to each product (Reactions S4.1 – S4.6 in the Supporting Information) and the number required to reduce MnO$_4^-$ to each of the three reduction products (Reactions S4.7 – S4.9 also in the Supporting Information). Note that these reactions are valid for any alkene substrate. Each pair of oxidation and reduction products result in a different number of electrons transferred from the substrate to MnO$_4^-$. As an example, the half-reaction for oxidation of
an alkene to two carboxylic acids is an 8 electron oxidation of the alkene substrate.

\[
\text{R} = \text{C} = \text{C} \quad \text{H} \quad + 4\text{H}_2\text{O} \quad \rightleftharpoons \quad 2\text{R} = \text{C} \quad \text{O} \quad \text{H} \quad + 8\text{H}^+ + 8\text{e}^- \quad \quad (4.10)
\]

The half-reaction for reduction of MnO\text{$_4$} to MnO$_2$ is a 3 electron reduction of MnO\text{$_4$}.

\[
\text{MnO}_4^- (\text{aq}) + 4\text{H}^+ (\text{aq}) + 3\text{e}^- \quad \rightleftharpoons \quad \text{Mn}^{\text{IV}}\text{O}_2 (\text{s}) + 2\text{H}_2\text{O} \quad \quad (4.11)
\]

From the balanced reaction the stoichiometry of moles of MnO\text{$_4$} consumed to moles of alkene consumed can be determined to be 2.67.

\[
3\quad \text{R} = \text{C} = \text{C} \quad \text{H} \quad + 8\text{MnO}_4^- + 8\text{H}^+ \quad \rightleftharpoons \quad 6\text{R} = \text{C} \quad \text{O} \quad \text{H} \quad + 8\text{MnO}_2 (\text{s}) + 4\text{H}_2\text{O} \quad \quad (4.12)
\]

Table 4.1 summarizes the balanced redox reactions by tabulating mole of MnO\text{$_4$} consumed per mole of alkene consumed for all combinations of organic oxidation products and inorganic reduction products. The balanced reactions are shown in the Supporting Information (Reactions S4.10 – S4.27).

The number moles of MnO\text{$_4$} required is greater when MnO$_2$ is product because it results from 3 electron reduction where MnO$_2$ results from 5 electron reduction. The largest value on Table 4.1 corresponds to two carboxylic acids and MnO$_2$ as the products, and the smallest is for the diol and Mn$^2+$ (aq). The high and low values correspond to the greatest and least extent of oxidation of alkene and most and least number of moles of MnO\text{$_4$} required to generated the products. In an actual reaction mixture the number of moles consumed per alkene will fall within this range.

In this study we employ cis-Stilbenedicarboxylic acid (SDCA) as a model alkene. SDCA is an ideal model compound because it is commercially available, it is at least somewhat
water soluble, it reacts on an observable time-scale, and it some of its oxidation products are commercially available or synthesizable. SDCA has carboxylic acid functional groups that make it and its expected $\text{MnO}_4^-$ oxidation products amenable to detection by liquid-chromatography and mass-spectrometry (LC/MS) with electrospray ionization.

Figure 4.1 shows the structure of SDCA and the anticipated $\text{MnO}_4^-$ oxidation products. Authentic standards are available for the $\text{C}=\text{C}$ double bond cleavage products: 4-carboxybenzaldehyde and terephthalic acid. Authentic standards are not available for the diol, ketol, and dione products, but peak areas can be reported from LC/MS analysis.

In this study, we show that SDCA can be used to study how solution conditions affect the final product distribution from $\text{MnO}_4^-$ oxidation of alkenes: in particular pH and the ratio of $\text{MnO}_4^-$ and SDCA concentration. We will compare the product distribution resulting from one set of solution conditions to another by analyzing the oxidation products of SDCA by LC/MS and by investigating Mn speciation using a modified iodometric titration method. We will conduct both time-course and reaction quench experiments to explore both kinetics and product distribution.

4.2 Materials and Methods

All aqueous solutions were prepared from reagent grade chemicals and distilled, deionized water (Milli-Q water, 18 M$\Omega$-cm resistivity, Millipore Corp., Milford, MA). All bottles and glassware were rinsed with distilled water, soaked in 5 M nitric acid overnight, rinsed with distilled water and Milli-Q water and then air-dried.
4.2.1 Chemicals

Ascorbic acid and 3-(N-morpholino)propanesulfonic acid (MOPS) were purchased from Sigma (St. Louis, MO). Sodium acetate, terephthalic acid, and benzoic acid were purchased from Aldrich (St. Louis, MO). 4,4′-cis-Stilbenedicarboxylic acid (SDCA), 4-carboxybenzaldehyde, and hydroxylamine hydrochloride were purchased from Alfa Aesar (Pelham, NH). NaCl was purchased from Acros Organics (Fair Lawn, NJ). HCl and LC/MS Optima grade methanol were purchased from Fisher Scientific (Pittsburgh, PA). Potassium permanganate (KMnO₄), sodium hydrogen carbonate (NaHCO₃), and ammonium acetate were purchased from Fluka (Buchs, Switzerland). Sodium thiosulfate (Na₂S₂O₃), sodium hydroxide (NaOH), potassium iodide (KI), dibasic anhydrous sodium phosphate (Na₂HPO₄), and acetic acid were purchased from J. T. Baker (Phillipsburg, NJ).

4-Carboxybenzaldehyde oxime was synthesized using the following procedure. An excess of hydroxylamine hydrochloride (2 g) was added to a continuously stirred solution of 4-carboxybenzaldehyde (0.545 g) dissolved in 100 mL of 0.1 M NaOH in an Erlenmeyer flask. The solution was left to react for 24 hrs. Then, 5 M HCl was added to lower the pH below 3, where the oxime product precipitates but unreacted hydroxylamine remains dissolved. Crystals of the oxime were collected by vacuum filtration, washed with 0.1 M HCl, and rinsed with Milli-Q water, and allowed to air dry until the mass of crystals and filter paper no longer decreased due to evaporation of water. This method gave 0.431 g of product or 86% yield. LC/MS analysis of a 50 µM aqueous solution of the product crystals yielded one peak with m/z corresponding to 4-carboxybenzaldehyde oxime.
4.2.2 Reaction Medium and Experimental Design

All experiments were performed in 100 mL polypropylene bottle reactors placed in a 25 ± 1°C constant temperature bath. Reaction solutions were continuously stirred with Teflon-coated stir bars and sparged with N₂(g). The N₂(g) sparge line is bubbled through water before reaching the experimental solution to minimize evaporation of the solution. Before every experiment, fresh stock solutions of SDCA, KMnO₄, NaCl, and the buffer of interest were prepared in Milli-Q water. Solutions contained 5.0 mM acetate to maintain pH between 4.0 and 5.0, 5.0 mM phosphate at pH 7.0, and 5.0 mM carbonate at pH 10.0. NaCl was added to set the ionic strength. For comparison purposes, some pH 7.0 experiments were performed without added buffer. Instead HCl and NaOH additions were used to maintain pH. All buffer stock solutions were adjusted to desired pH with NaOH or HCl before adding to reactors.

A quenching method was used because the reaction between SDCA and MnO₄²⁻ was too fast to be observed directly using LC/MS. Hydroxylamine (NH₂OH) reduces MnO₄⁻ to Mn²⁺ (60), which quenches the samples before they are analyzed by LC/MS. NH₂OH is oxidized by MnO₄⁻ to nitrite and nitrous oxide in strongly alkaline solution, nitric acid in strong acidic solution, and nitrogen gas under “weakly alkaline” conditions (60). Samples were taken by pipetting a 1 mL aliquot from the reactor to an autosampler vial containing 100 µL of “quench solution.” The quench solution consisted of 11.3 mM hydroxylamine, 300 µM benzoic acid, and 111 mM phosphate buffer adjusted to pH 7.0 and was prepared freshly before each experiment. The vials were shaken vigorously and analyzed by LC/MS. Control experiments showed that SDCA and terephthalic acid did not react with the agents in the quenching solution. Concentrations and peak areas detected by LC/MS did not change after quenching for up to two days of storage time. Benzoic acid serves as an LC/MS
internal standard. Hydroxylamine reacts with 4-carboxybenzaldehyde to form an oxime; the oxime was detected by LC/MS using a synthesized standard. No 4-carboxybenzaldehyde was detected in any experimental sample indicating that it was completely derivatized to the oxime. For simplicity, the concentration of 4-carboxybenzaldehyde oxime will be reported as 4-carboxybenzaldehyde from here on.

4.2.3 Time Course Experiments and Kinetic Analysis

A series of time course experiments was performed using solutions of SDCA, NaCl, and buffer of interest which were prepared as described above. The stock of MnO$_4^-$ was added last to initiate reaction. Samples were taken periodically, quenched, and analyzed by LC/MS. No concentration data was collected for MnO$_4^-$ or other Mn compounds. The concentration of SDCA, terephthalic acid, and 4-carboxybenzaldehyde were determined by LC/MS. The concentration of the ketone, diol, and dione products were determined by considering the mass balance. The following mass balance expression equates the SDCA initially added to the solution ($[SDCA]_0$) to the sum of any SDCA remaining at the time of quenching and the oxidation products.

$$[SDCA]_0 = [SDCA] + 0.5[4\text{-carboxybenzaldehyde}] +$$

$$0.5[\text{terephthalic acid}] + [\text{ketol}] + [\text{dione}] + [\text{diol}] \quad (4.13)$$

4-Carboxyaldehyde and terephthalic acid arise from cleavage of the carbon-carbon double bond in SDCA. Because SDCA is symmetric, two molecules of 4-carboxybenzaldehyde are generated for each SDCA that is cleaved. The stoichiometric ratio is represented by the coefficient of 0.5 in Equation 4.13. A coefficient of 0.5 is also required for terephthalic acid because of the one-to-one stoichiometry of 4-carboxybenzaldehyde oxidation to terephthalic
acids. LC/MS analysis of calibration standards of SDCA, 4-carboxybenzaldehyde, and terephthalic acid demonstrate that concentration was linearly related to peak area. For these three compounds, the slope of the calibration curve was determined using the standards. Equations 4.14 – 4.16 assume that concentration is linearly related to peak area for the ketol, dione, and diol oxidation products.

\[
\text{ketol} = m_{\text{ketol}}A_{\text{ketol}} \tag{4.14}
\]

\[
\text{dione} = m_{\text{dione}}A_{\text{dione}} \tag{4.15}
\]

\[
\text{diol} = m_{\text{diol}}A_{\text{diol}} \tag{4.16}
\]

where \( m_i \) is the slope of the calibration curve for compound \( i \) and \( A_i \) is the peak area for \( i \).

Substituting Equations 4.14 – 4.16 into Equation 4.13 gives

\[
[\text{SDCA}]_0 = [\text{SDCA}] + 0.5[4\text{-carboxybenzaldehyde}] + 0.5[\text{terephthalic acid}] + m_{\text{ketol}}A_{\text{ketol}} + m_{\text{dione}}A_{\text{dione}} + m_{\text{diol}}A_{\text{diol}} \tag{4.17}
\]

Equation 4.17 can be used to determine best-fit values of each slope by nonlinear least-squares regression where the three slopes are the fitting parameters. The slopes were assumed to be constant for all samples analyzed on the same day. Duplicate analysis of standards for SDCA, 4-carboxybenzaldehyde, and terephthalic acid show that the slopes for these compounds do not change significantly when samples are analyzed on the same day. It was assumed that the slopes for ketol, dione, and diol would also be consistent within the same day. A system of equations was set up consisting of a version of Equation 4.17 for each sample collected and analyzed on the same day by incorporating concentration data for SDCA, 4-carboxybenzaldehyde, and terephthalic acid and peak area of ketol, dione, and
diol. Optimal values for each slope were determined using the Solver tool within Microsoft Excel (61). Values were selected that most closely achieved mass balance and that, were all greater than or equal to zero, and that resulted in concentrations of ketol, dione, and diol that made physical sense.

In a separate experiment, the kinetics of MnO$_4^-$ oxidation of 4-carboxybenzaldehyde to terephthalic acid were investigated using solutions prepared identically to the solutions above, except that the substrate was changed from SDCA to 4-carboxybenzaldehyde. Concentrations of 4-carboxybenzaldehyde and terephthalic acid were monitored using the hydroxylamine quenching method and LC/MS. Pseudo-first-order kinetic modeling was used to determine the rate constant because the concentration of MnO$_4^-$ was more than an order of magnitude greater than the concentration of 4-carboxybenzaldehyde.

4.2.4 Reaction Quench Experiments and Oxidizing Equivalents

A second series of experiments was conducted where reaction was initiated by MnO$_4^-$ addition and allowed to proceed for 30 min in order to test product distribution and reaction stoichiometry under varying pH conditions. Reaction solutions were prepared as above. Following the reaction time, one 100 µL aliquot of the reaction slurry was quenching solution as described above and analyzed by LC/MS determine concentration of SDCA, terephthalic acid, 4-carboxybenzaldehyde, and peak areas of ketol, diol, and dione. Iodometric titration (62) was adapted to determine the number of oxidizing equivalents (OE) in an unfiltered and filtered aliquot, where OE is the number of moles of electrons per liter of sample slurry or solution obtained by reducing all oxidants to their fully reduced forms by addition of a reductant. Iodometric titration has been used previously to determine the average oxidation state of Mn$^{III,IV}$ (hydr)oxide solids (63). Here, iodide was used to reduce MnO$_4^-$. 

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Mn\textsuperscript{IV}, and Mn\textsuperscript{III} to Mn\textsuperscript{2+}, which also quenches the sample. Concentrations or loadings of Mn\textsuperscript{VI} and Mn\textsuperscript{V} were assumed to be negligible. Reaction of MnO\textsubscript{4}\textsuperscript{−} in excess iodide (I\textsuperscript{−}) at acidic pH proceeds quantitatively to Mn\textsuperscript{2+} (64, 65). I\textsuperscript{−} is oxidized to iodine (I\textsubscript{2}), which forms triiodide ion (I\textsubscript{3}\textsuperscript{−}) in the presence of excess I\textsuperscript{−} (64). Hypoiodous acid (HOI) and periodate (IO\textsubscript{4}\textsuperscript{−}) can also be products of MnO\textsubscript{4}\textsuperscript{−} oxidation of I\textsuperscript{−}, but excess I\textsuperscript{−} and acidic pH are expected to favor I\textsubscript{3}\textsuperscript{−} (64). In tests, no change was detected in the concentration of SDCA, 4-carboxybenzaldehyde, terephthalic acid or peak area of ketol, diol, or dione that resulted from any step of iodometric analysis that could not be accounted for by dilution.

Iodometric titration was performed on 2 aliquots of each reaction slurry. The analysis of samples is depicted as a flow chart in the supporting information (Figure S4.3). For the first aliquot, approximately 15 mL of slurry were filtered into a flask using a 0.2 \( \mu \)m pore diameter track-etched polycarbonate filter membranes (Whatman, UK). A 10 mL aliquot of this filtered solution was pipetted into an Erlenmeyer flask. This aliquot served as the filtered sample. A second 10 mL aliquot of the reaction slurry was pipetted into a second Erlenmeyer flask and served as the unfiltered sample. To each flask, 500 \( \mu \)L of 1 M HCl and 0.5 gram of KI was added, yielding a yellow or brown solution. The resulting solutions were titrated with 1 mM sodium thiosulfate (Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}). Just before reaching the colorless endpoint, several drops of 1 \% starch solution were added resulting in a blue solution. More Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} titrant was added until the endpoint was reached.

OE is related to concentration of MnO\textsubscript{4}\textsuperscript{−}, Mn\textsuperscript{IV}, and Mn\textsuperscript{III} by the number of electrons transferred in the half-reaction representing reduction to Mn\textsuperscript{2+}:

\[
\text{MnO}_4^- (aq) + 8H^+ (aq) + 5e^- \rightleftharpoons \text{Mn}^{2+} (aq) + 4H_2O \quad (4.18)
\]

\[
\text{Mn}^{IV}O_2(s) + 4H^+ (aq) + 2e^- \rightleftharpoons \text{Mn}^{2+} (aq) + 2H_2O \quad (4.19)
\]
\[
\text{Mn}^{\text{III}}\text{OOH}(s) + 3\text{H}^+(aq) + e^- \rightleftharpoons \text{Mn}^{2+}(aq) + 2\text{H}_2\text{O} \quad (4.20)
\]

Before any reaction with SDCA occurs, all of the OE are present as MnO$_4^-$. Initial OE (OE$_0$) can be calculated from $[\text{MnO}_4^-]_0$:

\[
\text{OE}_0 = 5[\text{MnO}_4^-]_0 \quad (4.21)
\]

Also consider the Mn mass balance equation:

\[
\text{Mn}_\text{TOT} = [\text{MnO}_4^-]_0 = [\text{MnO}_4^-] + [\text{Mn}^{\text{IV}}] + [\text{Mn}^{\text{III}}] + [\text{Mn}^{2+}] \quad (4.22)
\]

where Mn$_\text{TOT}$ is the total amount of manganese present in the slurry. Combining Equations 4.21 and 4.22 gives the following expression for OE initially present in the solution:

\[
\text{OE}_0 = 5[\text{MnO}_4^-]_0 = 5[\text{MnO}_4^-] + 5[\text{Mn}^{\text{IV}}] + 5[\text{Mn}^{\text{III}}] + 5[\text{Mn}^{2+}] \quad (4.23)
\]

The OE measured in the unfiltered sample represents residual MnO$_4^-$, and any Mn$_{IV}$ or Mn$_{III}$ in the suspension

\[
\text{OE(unfiltered)} = 5[\text{MnO}_4^-] + 2[\text{Mn}^{\text{IV}}] + [\text{Mn}^{\text{III}}] \quad (4.24)
\]

where OE(unfiltered) is the number of oxidizing equivalents determined by iodometric titration of the unfiltered sample. The coefficients in Equation 4.24 arise from Equations 4.18, 4.19, and 4.20. In the filtered sample, the measured OE are assumed to arise from
MnO$_4^-$. The residual MnO$_4^-$ concentration can be calculated using Equation 4.25.

\[ OE(\text{filtered}) = 5[MnO_4^-] \]  

(4.25)

Combining Equations 4.21 and 4.25 yields the amount of MnO$_4^-$ (in concentration units) that had been consumed by the quench time:

\[ \text{MnO}_4^- \text{ (consumed)} = \frac{OE_0 - OE(\text{filtered})}{5} \]  

(4.26)

Subtracting OE(filtered) from OE(unfiltered) gives the OE from Mn$^{\text{III}}$ and Mn$^{\text{IV}}$ particles that are trapped by the filter.

\[ OE(\text{particulate}) = OE(\text{unfiltered}) - OE(\text{filtered}) = 2[Mn^{\text{IV}}] + [Mn^{\text{III}}] \]  

(4.27)

Finally, subtracting Equation 4.24 from Equation 4.23 gives the number of OE that were transferred during the allotted reaction time.

\[ OE(\text{transferred}) = OE_0 - OE(\text{unfiltered}) = 3[Mn^{\text{IV}}] + 4[Mn^{\text{III}}] + 5[Mn^{2+}] \]  

(4.28)

This method has some limitations. The OE assigned to Mn$^{\text{III}}$ and Mn$^{\text{IV}}$ depend on the filter cutoff size. Thus OE(particulate) can only be operationally defined and would not account for any particulates that pass through the filter. Other solution constituents can stabilize or destabilize the Mn$^{\text{III,IV}}$ (hydr)oxide solid particles against aggregation impacting whether or not they are removed by filtration. For example, pyrophosphate can chelate Mn$^{\text{III}}$ (66) forming a dissolved complex that would pass through the filter. Any Mn$^{\text{III}}$ and Mn$^{\text{IV}}$ that pass through the filter would be reported as MnO$_4^-$. 

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Electron equivalents of SDCA oxidized can be calculated by considering the stoichiometry and number of electrons transferred in each half-reaction shown in Table 4.1 and the product calculations determined using Equation 4.17. These equivalents will be called reducing equivalents (RE) to contrast them with OE described above.

\[
\text{RE(transfered)} = 4[\text{terephthalic acid}] + 2[\text{4-carboxybenzaldehyde}] + 6[\text{dione}] + 4[\text{ketol}] + 2[\text{diol}]
\]  

(4.29)

### 4.2.5 Instrumental Analysis

Concentrations of the SDCA, 4-carboxybenzaldehyde oxime, and terephthalic acid as well as peak areas of ketol, diol, and dione oxidation products were determined by LC/MS. Benzoic acid served as an internal standard. The LC/MS system consisted of a Waters 2795 Alliance separation module and a Micromass Quattro Micro Triple Quadrupole Mass Spectrometer (Waters, Milford, MA). Separation was achieved using a Waters Atlantis T3 column (4.6 x 100 mm, 5 µm particle size). Gradient elution at a flow rate of 1 mL min\(^{-1}\) was used where mobile phase A was 0.2% v/v acetic acid adjusted to pH 4.0 by addition of ammonium hydroxide and mobile phase B was methanol. The gradient run method was: 0–6 min 10% B, 6–9 min increase to 80% B, and 9–9.5 min decrease to 10% B. The mass spectrometer was equipped with an electrospray ion source, which was operated in negative mode. All compounds were detected based on their molecular ions ([M-H]\(^{-}\)). The cone voltage setting was between -25 and -15 V depending on the analyte. Single ion recording mode was used to detect each compound set to the mass to charge ratio (m/z) of the molecular ion, the values used for SDCA and each product are given in Figure 4.1.
4.3 Results

4.3.1 Time Course Experiments

Hydroxylamine quenching made it possible to investigate the kinetics of 20 μM SDCA oxidation by MnO$_4^-$ (Figure 4.2). A MnO$_4^-$ concentration of 200 μM lead to complete consumption of SDCA within 10 minutes of reaction. At 20 μM MnO$_4^-$, only 15 μM of SDCA was consumed; 5 μM SDCA remained after 1 hour of reaction. Any effects of buffer identity or pH (performed at pH 5.0, 7.0, and 10) were too small to discern using the quenching technique. In control experiments lacking MnO$_4^-$, no loss of SDCA was detected at pH 7.0 (with or without 5 mM phosphate buffer) or pH 10.0 (5 mM carbonate buffer); however, at pH 5.0 (5 mM acetate buffer) approximately 18% loss of this model alkene was observed (Figure S4.4 in the Supporting Information).

Terephthalic acid, 4-carboxybenzaldehyde, ketol and dione were detected in solutions of 20 μM SDCA 200 μM MnO$_4^-$ in 5 mM acetate (pH 5.0) buffered solution with ionic strength adjusted to 10 mM with NaCl (Figure 4.3). As in Figure 4.2, SDCA was consumed in less than 10 minutes. LC/MS evidence suggests that aldehyde, acid, ketol, and dione are the only significant products at pH 5.0. Aldehyde and ketol oxidation products reach a maximum concentration between 5 and 10 min then decrease somewhat over the remaining contact time. After 1 hour, aldehyde and ketol were the major products. The concentration of dione and carboxylic acid products increased gradually over time.

The hydroxylamine quench method was also used to monitor loss of 20 μM SDCA with increasing the concentration of MnO$_4^-$ (Figure 4.4). These solutions also contained 5.0 mM acetate adjusted to pH 4.0 and 10 mM ionic strength adjusted by addition of NaCl. SDCA was completely consumed within 11 min at MnO$_4^-$ concentrations greater than 170 μM. The
dependence of aldehyde, ketol, and acid product concentration on initial concentration of MnO$_4^-$ was complex. For ketol and aldehyde, the 11 min concentration was greater at 170 and 85 µM Mn$_{\text{04}}$ than at 42 or 218 µM Mn$_{\text{04}}$. For terephthalic acid, both the initial rate of generation and the 11 min concentration did not change with increasing concentration of MnO$_4^-$, except that a slightly higher 11 min concentration was detected when [MnO$_4^-$]$_0$ was 85 µM than at the other concentrations tested.

In Figure 4.3, only 2.5 µM of terephthalic acid was detected after 60 min, which is consistent with a rate of oxidation of the 4-carboxybenzaldehyde to terephthalic acid that is significantly slower than the rate of oxidation of SDCA. We can directly observe the rate of this reaction experimentally because authentic standards are available for both the 4-carboxybenzaldehyde and terephthalic acid. We monitored the loss of 4-carboxybenzaldehyde and generation of terephthalic acid with [Mn$^+_4$]$_0$ of 200 and 400 µM in 5 mM acetate (pH 5.0) with ionic strength adjusted to 10 mM using NaCl (Figure 4.5). When [MnO$_4^-$]$_0$ was 200 µM (as in Figure 4.3) the half-life was approximately 160 min. Doubling [MnO$_4^-$]$_0$ to 400 µM shortened the half-life to 80 min. The rate constant was determined to be $k = 3.6 \pm 0.6 \times 10^{-1}$ M$^{-1}$ s$^{-1}$ by regression using a pseudo-first-order kinetic model. Excellent mass balance was maintained throughout both experiments. In a separate experiment, 400 µM MnO$_4^-$ and 20 µM terephthalic acid were added to a solution containing 5.0 mM acetate buffer (pH 5.0) with ionic strength adjusted to 10.0 mM using NaCl. No loss of terephthalic acid was observed using the quenching method over 5 hours of reaction.
4.3.2 Reaction Quench Experiments and Oxidizing Equivalents

Concentrations of residual MnO$_4^-$ and the Mn$^{III,IV}$ solids and Mn$^{2+}$(aq) generated after 30 min was measured using iodometry in a series of experiments employing SDCA. In Figure 4.3B, manganese speciation was determined for one time point in the reaction, at 30 minutes. When [MnO$_4^-$]$_0$ is 200 µM, the total number of oxidizing equivalents added is 1 mEq L$^{-1}$. After 30 min, OE(filtered) was 710 µEq L$^{-1}$. The filtered sample corresponds to 142 µM MnO$_4^-$ meaning that 58 µM of MnO$_4^-$ was consumed after 30 min. OE(particulate), representing Mn$^{III}$ and Mn$^{IV}$ particulates removed by filtration, accounted for 199 µEq L$^{-1}$. The remaining oxidizing equivalents 91 µEq L$^{-1}$ were transferred during MnO$_4^-$ oxidation of SDCA.

MnO$_4^-$ was added to 20 µM of SDCA solutions and were quenched after 30 min. Manganese speciation and organic product yield are shown in Figure 4.6, and in tabular form in the supporting information (Table S4.3). Five buffer systems were employed: 5.0 mM acetate (pH 5.0), 5.0 mM phosphate (pH 7.0), 5.0 mM pyrophosphate (pH 7.0), pH 7.0 maintained by adding small amounts of HCl or NaOH (termed “unbuffered”), and 5.0 mM carbonate (pH 10). There was significant OE(particulate) measured in the pH 5.0 buffer and at pH 7 in the absence of buffer, but not in the pH 10.0 buffer or at pH 7.0 in either phosphate and pyrophosphate buffer. No OE(filtered) was detected in pH 5.0 and pH 7.0 (unbuffered) solutions when [MnO$_4^-$]$_0$ was 20 µM or less. At the 30 minute quench time, pH 5.0, pH 7.0 (unbuffered) and pH 7.0 (5 mM phosphate) samples were brown, but pH 7.0 (5 mM pyrophosphate) and pH 10.0 were red.

Aldehyde and ketol were the major organic oxidation products detected at the 30 minute quench time for all pH and buffer systems tested (Figure 4.6). Figure 4.6 shows the products by contribution to mass balance based on Equation 4.13, these data are shown as concen-
Regardless of pH and buffer identity, all SDCA was consumed at \([\text{MnO}_4^-]_0\) greater than 40 \(\mu\text{M}\). The 30 minute concentrations of ketol and 4-carboxybenzaldehyde were the largest in solutions with 40 \(\mu\text{M}\) initial concentration of \(\text{MnO}_4^-\). At \([\text{MnO}_4^-]_0\) greater than 40 \(\mu\text{M}\), the 30 minute concentration of 4-carboxybenzaldehyde decreased with increasing \([\text{MnO}_4^-]_0\), with the exception of the absence of buffer at pH 7.0. Ketol concentration did not decrease at \([\text{MnO}_4^-]_0\) higher than 40 \(\mu\text{M}\). Terephthalic acid was detected in all buffer systems, but the concentration did not exceed 3.5 \(\mu\text{M}\). No significant concentration of the dione or diol was detected.

OE\text{(transferred)} and RE\text{(transferred)} after 30 minutes of contact time are compared in Table 4.2. The values of OE\text{(transferred)} are typically similar or larger than RE\text{(transferred)} at high concentrations of \(\text{MnO}_4^-\). RE\text{(transferred)} was larger than OE\text{(transferred)} at low \(\text{MnO}_4^-\).

Regardless of pH the ratio of \(\text{MnO}_4^-\) consumed to SDCA consumed increased with increasing concentration of \(\text{MnO}_4^-\) (Figure 4.7A) except for the solution adjusted to pH 7.0 without a buffer with 10 \(\mu\text{M}\) initial concentration of \(\text{MnO}_4^-\). The presence of 5.0 mM carbonate (pH 10.0) or 5.0 mM pyrophosphate (pH 7.0) kept the \(\text{MnO}_4^-\) to SDCA consumption ratio near 1, but the ratio was higher for pH 7.0 (5.0 mM phosphate), pH 5.0 (5.0 mM acetate), and pH 7 in the absence of a buffer (Figure 4.7), with no ligand present some \(\text{MnO}_4^-\) was reduced to \(\text{Mn}^{\text{III}}\text{IV}\) solids. Solid formation was also favored at pH 5.0 (Figure 4.6).

The ratio of OE\text{(transferred)} to SDCA consumed (Figure 4.7B) also generally increased with increasing concentration of \(\text{MnO}_4^-\). The unbuffered pH 7.0 solution had the highest values for both ratios. The 5.0 mM acetate (pH 5.0) solution had the second highest values.
for the ratio of MnO$_4^-$ consumed to SDCA consumed (Figure 4.7A), but the lowest values for the ratio of OE(transferred) to SDCA consumed (Figure 4.7B).

### 4.4 Discussion

We expect that oxidation of SDCA by MnO$_4^-$ occurs in a series of steps. The first step is formation of an intermediate that we could not detect. The first intermediate is expected to be the cyclic Mn$^V$ intermediate shown in Figure 4.1. The second step is one of two reactions: the reaction that generates 4-carboxybenzaldehyde from the intermediate or the reaction that generates ketol from the intermediate.

If 4-carboxybenzaldehyde is the product of the second step, then the third step is oxidation of 4-carboxybenzaldehyde to terephthalica acid. For ketol, the third step is oxidation of ketol to dione. We will examine how our experimental results support this proposed scheme and consider the effect of pH and the concentration of MnO$_4^-$. Solution pH did not influence the rate of the first step, Figure 4.2, at both initial concentrations of MnO$_4^-$. The lack of pH effect on substrate consumption rate was also reported for trichloroethylene (10) and carbamazepine (21). MnO$_4^-$ has a pK$_a$ of MnO$_4^-$ is $-2.24$ (67) and exists as an anion at all pH we tested.

The first proposed reaction step involves both SDCA and MnO$_4^-$ and accordingly the rate of SDCA consumption increased with increasing concentration of MnO$_4^-$ (Figure 4.2 and Figure 4.4). However, the stoichiometry is greater than one to one: on the time scales we considered (less than 60 minutes) 20 µM SDCA was not completely consumed in solutions of 20 µM MnO$_4^-$. MnO$_4^-$ is expected to be involved in the second and third steps, which could consume MnO$_4^-$ before SDCA is completely consumed.
The consumption of SDCA, generation of 4-carboxybenzaldehyde and generation of ketol represent reactions that generate and consume the first intermediate. In Figure 4.3A the concentration of SDCA fell below the detection limit in less than 10 min while the aldehyde and ketol oxidation products reached their highest concentration on roughly the same time scale. These similar rates of generation and consumption are indicative of the short-lived nature of the cyclic Mn$^V$ intermediate.

We would expect that the rates of the two possible reactions that happen in the second step (either oxidation of the cyclic Mn$^V$ intermediate to either ketol or two molecules of 4-carboxybenzaldehyde) would also increase with increasing MnO$_4^-$ concentration. However, Figure 4.4 contains peculiar results for 4-carboxybenzaldehyde and ketol. The highest MnO$_4^-$ concentration did not yield the highest 11 minute concentration of ketol, 4-carboxybenzaldehyde, or terephthalic acid. Perhaps 4-carboxybenzaldehyde and ketol are quickly oxidized at the highest concentration of MnO$_4^-$ at pH 4.0, but this was not observed at pH 5.0 (Figure 4.3). Significantly higher concentration of terephthalic acid was observed at pH 4.0 (Figure 4.4) than at any other pH we tested (Figure 4.6). We speculate that low pH would promote terephthalic acid generation. Toland et al. (68) reported that oxidation of SDCA with permanganate yielded terephthalic acid “almost quantitatively.”

Solution pH did not significantly influence the distribution of organic products (Figure 4.6). Perhaps pH outside of the range 5.0 < pH < 10.0 would be required to observe a significant influence of pH on both the reaction rate and the distribution of products.

The subsequent oxidation steps leading to final products may be much slower than oxidation of SDCA. The rate of 4-carboxybenzaldehyde oxidation depends on the initial concentration of MnO$_4^-$ (Figure 4.5). This is reflected in the decrease of 4-carboxybenzaldehyde concentration and increase of Terephthalic acid concentration at initial MnO$_4^-$ concentrations
greater than 40 μM: In Figure 4.6, increasing the concentration of MnO$_4^-$ lead to greater oxidation of 4-carboxybenzaldehyde to terephthalic acid.

In our proposed scheme, 4-carboxybenzaldehyde is generated from the cyclic intermediate and consumed by oxidation to terephthalic acid. The generation step is fast, leading to a maximum concentration in 3.5 minutes in Figure 4.3. After reaching a maximum, the 4-carboxybenzaldehyde concentration slowly decreases which we attribute to oxidation of 4-carboxybenzaldehyde. The concentration of 4-carboxybenzaldehyde decreases by 1.85 μM between the maximum and 60 minutes. Over the same time period (3.5 to 60 minutes), terephthalic acid concentration increases by 1.54 μM. The close agreement in these values is consistent with the slow oxidation of 4-carboxybenzaldehyde to terephthalic acid, which we also observed directly in solutions of 4-carboxybenzaldehyde and MnO$_4^-$. Concentrations of 4-carboxybenzaldehyde in 30 minute quench experiments (Figure 4.6) also reflect that the oxidation of 4-carboxybenzaldehyde by MnO$_4^-$ to terephthalic acid is slow and dependent on the concentration of MnO$_4^-$. Regardless of pH and buffer identity, only small concentrations of terephthalic acid were observed after 30 minutes. The concentration of 4-carboxybenzaldehyde in Figure 4.6 decreased at high concentrations of MnO$_4^-$, consistent with more oxidation to terephthalic acid in the same time period. If more contact time had been allowed, we would expect larger concentration of terephthalic acid and lower concentration of 4-carboxybenzaldehyde.

The time course for ketol was similar to 4-carboxybenzaldehyde consistent with fast generation of ketol of the cyclic Mn$^V$ intermediate and slow consumption via oxidation to dione (Figure 4.3). We could not perform an experiment to determine the rate constant of ketol oxidation to dione because of the lack of authentic standards. We hypothesize that this oxidation reaction is similarly slow. We can only base that hypothesis on Figure 4.3
because no significant concentration of dione was observed in the 30 minute quenching data Figure 4.6.

At pH 5.0 and order-of-magnitude excess of MnO$_4^-$ in Figure 4.3, the amount of MnO$_4^-$ consumed was almost triple the initial concentration of SDCA (Figure 4.2B). When MnO$_4^-$ is in excess, we expect that the stoichiometry will be higher than 1:1 because of MnO$_4^-$ oxidation of the intermediate and some of the products. The 200 OE that remain as solids may have come from the 4 electron oxidation of SDCA to aldehyde or ketol: the corresponding 4 electron reduction of MnO$_4^-$ would give Mn$^{\text{III}}$ as the product (S4.8).

Solutions buffered with 5 mM phosphate or pyrophosphate at pH 7.0 suppressed the Mn$^{\text{III,IV}}$ solids that were detected in the absence of a buffer (Figure 4.6). Both phosphate and pyrophosphate are known to adsorb strongly to oxide surfaces, which could prevent aggregation resulting in particles small enough to pass through the filter. Jiang et al. (19, 69) confirmed the presence of a Mn$^{\text{III}}$ pyrophosphate species generated during MnO$_4^-$ oxidation of phenolic endocrine disrupting compounds. Pyrophosphate chelates Mn$^{\text{III}}$ rendering it soluble and stable, preventing disproportionation to MnO$_2$(s) and Mn$^{2+}$(aq) (50). Phosphate should exhibit the same effect, albeit to a lesser extent.

Higher initial concentration of MnO$_4^-$ favors more highly oxidized products. Oxidation of aldehyde to acid consumes MnO$_4^-$ without affecting SDCA, causing the ratio in Figure 4.7A to increase. Mn$^{\text{III,IV}}$ solids are products of 3 or 4 electron reduction of MnO$_4^-$ (Equations S4.8 and S4.7), while Mn$^{2+}$ is the 5 electron reduction product (Equation S4.9). Under conditions where no solid formed, each MnO$_4^-$ consumed transferred 5 e$^-$ to the reductants. Each MnO$_4^-$ that is reduced to Mn$^{\text{III}}$ or Mn$^{\text{IV}}$ only transfers 3 or 4 electrons, so more MnO$_4^-$ must be reduced to achieve the same amount of electron transfer. The extent of oxidation of SDCA and generation of products was similar regardless of pH and buffer identity
(Figure 4.6). To achieve the same extent of oxidation when Mn$_{\text{III,IV}}$ solids are generated more MnO$_4^-$ must be consumed, increasing the ratio shown on Figure 4.7.

Ideally, OE(transferred) and RE(transferred) would be equal and confirm that the number of electrons that were transferred from SDCA via oxidation equaled the number of electrons transferred to MnO$_4^-$ via reduction (Table 4.2). The discrepancy between the two could reflect limitations in the iodometric method. Although we did not observe any other peaks using LC/MS that would correspond to other oxidation products, any undetected product that is oxidized by MnO$_4^-$ would increase OE(transferred) but would not be accounted for in RE(transferred).

### 4.5 Conclusions

Using SDCA as a model alkene with a hydroxylamine quench model and modified iodometry, we have demonstrated a means monitoring manganese speciation and the identity and yields of organic oxidation products. Oxidation of SDCA is much faster than the oxidation of the aldehyde containing products to the corresponding carboxylic acids. Aldehydes and ketols are the major products of SDCA oxidation by MnO$_4^-$. Carboxylic acids and diones may be significant products on longer time scales at high doses of MnO$_4^-$. Within the range $5 < \text{pH} < 10$, pH had a significant effect, but the identity of the buffer had a less pronounced effect. Nonzero RE(transferred) in the absence of MnO$_4^-$ may indicate that some other oxidant, likely O$_2$, can oxidize SDCA and generate the same products.
References


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(61) Microsoft Excel for Mac 2011. **2010**.


Table 4.1. Moles of MnO$_4^-$ consumed per mol of alkene consumed required to generate specific organic oxidation and inorganic reduction products based on balanced redox reactions. Half-reactions are shown for oxidation of an alkene substrate. The number of electrons required to reduce MnO$_4^-$ to each reduction product are shown. Balanced redox reactions are shown in 4.6.1 in the Supporting Information.

<table>
<thead>
<tr>
<th>Alkene Oxidation Product Half-Reaction</th>
<th>MnO$_2$(s)</th>
<th>MnOOH(s)</th>
<th>Mn$^{2+}$(aq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R\text{CH}=$CH$\text{CH}=$R + 4 H$_2$O $\rightleftharpoons$ R$\text{C}=$OH + R$\text{C}$OH + 8 H$^+$ + 8 e$^-$</td>
<td>2.67</td>
<td>2</td>
<td>1.6</td>
</tr>
<tr>
<td>$R\text{CH}=$CH$\text{CH}=$R + 3 H$_2$O $\rightleftharpoons$ R$\text{C}=$OH + R$\text{C}$H$^+$ + 6 H$^+$ + 6 e$^-$</td>
<td>2</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>$R\text{CH}=$CH$\text{CH}=$R + 2 H$_2$O $\rightleftharpoons$ R$\text{C}$C$=$R + 6 H$^+$ + 6 e$^-$</td>
<td>2</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>$R\text{CH}=$CH$\text{CH}=$R + 2 H$_2$O $\rightleftharpoons$ R$\text{C}$C$=$R + 4 H$^+$ + 4 e$^-$</td>
<td>1.33</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>$R\text{CH}=$CH$\text{CH}=$R + 2 H$_2$O $\rightleftharpoons$ R$\text{C}$H$^+$ + R$\text{C}$H$^+$ + 4 H$^+$ + 4 e$^-$</td>
<td>1.33</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>$R\text{CH}=$CH$\text{CH}=$R + 2 H$_2$O $\rightleftharpoons$ R$\text{C}$H$^+$ + R$\text{C}$H$^+$ + 2 H$^+$ + 2 e$^-$</td>
<td>0.67</td>
<td>0.5</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Table 4.2. Comparison of equivalents of MnO$_4^-$ reduced, shown as OE(transferred), and equivalents of SDCA oxidized, shown as RE(transferred), measured after 30 minutes of contact time in buffered solutions of 20 µM SDCA and MnO$_4^-$ with ionic strength adjusted to 10 mM with NaCl.

<table>
<thead>
<tr>
<th>Buffer (pH)</th>
<th>[MnO$_4^-$]$_0$ (µM)</th>
<th>OE(transferred) (µEq L$^{-1}$)</th>
<th>RE(transferred) (µEq L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unbuffered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pH 7.0)</td>
<td>0</td>
<td>0</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>49</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>44</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>58</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>91</td>
<td>75</td>
</tr>
<tr>
<td>5.0 mM Acetate (pH 5.0)</td>
<td>0</td>
<td>0</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>49</td>
<td>48</td>
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<tr>
<td></td>
<td>40</td>
<td>44</td>
<td>70</td>
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<tr>
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<td>58</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>91</td>
<td>75</td>
</tr>
<tr>
<td>5.0 mM Phosphate (pH 7.0)</td>
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<td>0</td>
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</tr>
<tr>
<td></td>
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<td>26</td>
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<td></td>
<td>20</td>
<td>50</td>
<td>64</td>
</tr>
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<td>80</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>113</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>145</td>
<td>90</td>
</tr>
<tr>
<td>5.0 mM Pyrophosphate (pH 7.0)</td>
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<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>40</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>70</td>
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</tr>
<tr>
<td></td>
<td>40</td>
<td>90</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>103</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>120</td>
<td>85</td>
</tr>
<tr>
<td>5.0 mM Carbonate (pH 10.0)</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>31</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>60</td>
<td>58</td>
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<td>92</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>87</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>128</td>
<td>110</td>
</tr>
</tbody>
</table>
Figure 4.1. Molecular structure of cis-stilbenedicarboxylic acid (SDCA), proposed cyclic MnV intermediate, and products. Each product is labeled with the required number of electrons SDCA must be oxidized by to generate it and the mass-to-charge ratio (m/z) of the molecular ion used to detect it by electrospray mass spectrometry.
Figure 4.2. Loss of cis-stilbenedicarboxylic acid in the presence of MnO$_4^-$. Conditions: 20 µM SDCA, ionic strength adjusted to 10 mM with NaCl.
Figure 4.3. (A) cis-Stilbenedicarboxylic acid (SDCA) loss in the presence of 200 μM MnO$_4^-$.
Conditions: 20 μM SDCA, 5 mM acetate buffer (pH 5.0), and ionic strength adjusted to 10 mM using NaCl. The concentration of SDCA in a separate control experiment lacking MnO$_4^-$ is shown for comparison. (B) Results obtained from iodometric titration of filtered and unfiltered aliquots collected after 30 min of contact time.
Figure 4.4. Time course plots for reaction of 20 µM cis-Stilbenedicarboxylic acid (SDCA) and generation of 4-carboxybenzaldehyde, terephthalic acid and ketol in the presence of increasing amounts of MnO$_4^-$. The reaction medium contained 5.0 mM acetate (pH 4.0) with ionic strength adjusted to 10 mM using NaCl. The concentration of SDCA in a control experiment lacking MnO$_4^-$ is shown for comparison.
Figure 4.5. Time course plots for oxidation of 20 µM 4-carboxybenzaldehyde to terephthalic acid by (A) 200 µM MnO$_4^-$ and (B) 400 µM MnO$_4^-$. The reaction medium contained 5.0 mM acetate (pH 5.0) with ionic strength was adjusted to 10 mM using NaCl. Lines represent a pseudo-first-order model of 4-carboxybenzaldehyde consumption. Dashed lines a represent model of terephthalic acid generation. The sum of 4-carboxybenzaldehyde and terephthalic acid concentrations is shown as the mass balance.
Figure 4.6. Oxidizing equivalents (OE), unreacted cis-stilbenedicarboxylic acid (SDCA) and organic oxidation product concentrations after 30 min of contact time. Conditions: 20 µM SDCA, ionic strength adjusted to 10 mM using NaCl.
Figure 4.7. (A) Ratio of MnO$_4^-$ consumed to cis-stilbenedicarboxylic acid (SDCA) consumed and (B) ratio of OE(transferred) to SDCA consumed. Conditions: 20 µM SDCA, ionic strength adjusted to 10 mM using NaCl, reaction was quenched after 30 min of contact time.
4.6 Supporting Information

Supporting information for Chapter 4 includes half-reactions balanced reactions for redox reactions between alkenes and MnO$_4^-$ leading to the possible organic oxidation and inorganic reduction products that were referred to in Table 4.1, results from a series of experiments using 3-cyclopentene-1-carboxylic acid as a model alkene (4.6.2), a flow diagram of the sampling procedure for iodometry and LC/MS analysis in quenched experiments (Figure S4.3), time course results for control experiments showing loss of SDCA in the absence of MnO$_4^-$ (Figure S4.4), tabulated data shown on Figure 4.6, and a table of available acid dissociation constants (pK$_a$) for chemical compounds used in this study (Table S4.4).

4.6.1 Redox Reactions for Permanganate Oxidation of an Alkene

This section gives six half-reactions for oxidation of an alkene substrate and three for reduction of MnO$_4^-$. Following the half-reactions, eighteen balanced reactions are shown for each pair of alkene oxidation product and MnO$_4^-$ reduction product. These reactions were used to determine the stoichiometric ratios of MnO$_4^-$ consumed to alkene consumed shown in Table 4.1.

The half-reaction for two carboxylic acid final products requires 8 electron transfer overall from the alkene to MnO$_4^-$. 

$$ R\text{-CH=CH}\text{-R} + 4\text{H}_2\text{O} \rightleftharpoons R\text{-CO-}<sup>O</sup>\text{-OH} + R\text{-CO-}<sup>O</sup>\text{-OH} + 8\text{H}^+ + 8e^- \quad (S4.1) $$

190
If one carboxylic acid and one aldehyde are the final products then 6 electron transfer is required.

\[
\begin{align*}
R\text{CH\textendash\textendashCH\textendashCH\textendashR} + 3H_2O & \rightleftharpoons R\text{CO\textendashOH} + R\text{C\textendashH} + 6H^+ + 6e^- \quad (S4.2)
\end{align*}
\]

The half-reaction for dione formation also requires 6 electron transfer.

\[
\begin{align*}
R\text{CH\textendash\textendashCH\textendashCH\textendashR} + 2H_2O & \rightleftharpoons R\text{C\textendashC\textendashCH\textendashCH\textendashR} + 6H^+ + 6e^- \quad (S4.3)
\end{align*}
\]

Formation of the ketol product requires 4 \( e^- \) transfer.

\[
\begin{align*}
R\text{CH\textendash\textendashCH\textendashCH\textendashR} + 2H_2O & \rightleftharpoons R\text{CO\textendashOH} + R\text{CO\textendashH} + 4H^+ + 4e^- \quad (S4.4)
\end{align*}
\]

and so does forming two aldehydes.

\[
\begin{align*}
R\text{CH\textendash\textendashCH\textendashCH\textendashR} + 2H_2O & \rightleftharpoons R\text{C\textendashH}\text{\textendashCH\textendashC\textendashH} + 4H^+ + 4e^- \quad (S4.5)
\end{align*}
\]

Diol products result from only 2 electron transfer.

\[
\begin{align*}
R\text{CH\textendash\textendashCH\textendashCH\textendashR} + 2H_2O & \rightleftharpoons R\text{C\textendashH}\text{\textendashCH\textendashC\textendashH} + 2H^+ + 2e^- \quad (S4.6)
\end{align*}
\]

In summary, oxidative C\( =\)C double bond cleavage to two carboxylic acids requires transfer of 8 electrons, while one carboxylic acid and one aldehyde requires 6 electrons. Oxidation to a ketol product is a 4 electron transfer and the diol is a 2 electron transfer.

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Now, we will list the half-reactions for reduction of MnO$_4^-$.

The potential products of MnO$_4^-$ reduction are Mn$^{IV}$ solids, Mn$^{III}$ solids, and Mn$^{2+}$(aq). The half-reactions show that these products result from 3, 4, and 5 electron reductions, respectively.

\[
\text{MnO}_4^-(aq) + 4H^+(aq) + 3e^- \rightleftharpoons \text{Mn}^{IV}O_2(s) + 2H_2O \quad \text{(S4.7)}
\]

\[
\text{MnO}_4^-(aq) + 5H^+(aq) + 4e^- \rightleftharpoons \text{Mn}^{III}OOH(s) + 2H_2O \quad \text{(S4.8)}
\]

\[
\text{MnO}_4^-(aq) + 8H^+(aq) + 5e^- \rightleftharpoons \text{Mn}^{2+}(aq) + 4H_2O \quad \text{(S4.9)}
\]

The balanced redox reactions for all possible combinations of MnO$_4^-$ reduction products and alkene oxidation products are shown below. *Two Carboxylic Acids*

\[
\begin{align*}
3 \text{C} = \text{C}^\text{H} & + 8\text{MnO}_4^- + 8H^+ \rightleftharpoons 6\text{R} = \text{C}^\text{O} + 8\text{MnO}_2(s) + 4H_2O \quad \text{(S4.10)} \\
\text{R} = \text{C}^\text{H} & + 2\text{MnO}_4^- + 2H^+ \rightleftharpoons 2\text{R} = \text{C}^\text{O} + 2\text{MnOOH}(s) \quad \text{(S4.11)} \\
5 \text{C} = \text{C}^\text{H} & + 8\text{MnO}_4^- + 24H^+ \rightleftharpoons 10\text{R} = \text{C}^\text{O} + 8\text{Mn}^{2+} + 12H_2O \quad \text{(S4.12)}
\end{align*}
\]

*One Carboxylic Acid and One Aldehyde*

\[
\begin{align*}
\text{R} = \text{C}^\text{H} & + 2\text{MnO}_4^- + 2H^+ \rightleftharpoons \text{R} = \text{C}^\text{O} + \text{R} = \text{C}^\text{OH} + 2\text{MnO}_2(s) + H_2O \quad \text{(S4.13)}
\end{align*}
\]
\[
\begin{align*}
2 & \text{C} = \text{C}^+ + 3 \text{MnO}_4^- + 3 \text{H}^+ \rightleftharpoons 2 \text{R} = \text{C}^\text{O}^- + 2 \text{R} = \text{C}^- + 3 \text{MnOOH} \text{(s)} \\
5 & \text{C} = \text{C}^+ + 6 \text{MnO}_4^- + 18 \text{H}^+ \rightleftharpoons 5 \text{R} = \text{C}^\text{O}^- + 5 \text{R} = \text{C}^- + 6 \text{Mn}^{2+} + 9 \text{H}_2\text{O} \quad \text{(S4.14)}
\end{align*}
\]

**Dione**

\[
\begin{align*}
\text{R} = \text{C}^\text{O}^- + 2 \text{MnO}_4^- + 2 \text{H}^+ & \rightleftharpoons \text{R} = \text{C}^- + 2 \text{MnO}_2 \text{(s)} + 2 \text{H}_2\text{O} \\
2 & \text{C} = \text{C}^+ + 3 \text{MnO}_4^- + 3 \text{H}^+ \rightleftharpoons 2 \text{R} = \text{C}^\text{O}^- + 3 \text{MnOOH} \text{(s)} + 2 \text{H}_2\text{O} \\
5 & \text{C} = \text{C}^+ + 6 \text{MnO}_4^- + 18 \text{H}^+ \rightleftharpoons 5 \text{R} = \text{C}^\text{O}^- + 6 \text{Mn}^{2+} + 14 \text{H}_2\text{O} \quad \text{(S4.15)}
\end{align*}
\]

**Ketol**

\[
\begin{align*}
3 & \text{C} = \text{C}^+ + 4 \text{MnO}_4^- + 4 \text{H}^+ \rightleftharpoons 3 \text{R} = \text{C}^- + 4 \text{MnO}_2 \text{(s)} + 2 \text{H}_2\text{O} \\
\text{R} = \text{C}^\text{O}^- + \text{MnO}_4^- + \text{H}^+ & \rightleftharpoons \text{R} = \text{C}^- + \text{MnOOH} \text{(s)} \\
5 & \text{C} = \text{C}^+ + 4 \text{MnO}_4^- + 12 \text{H}^+ \rightleftharpoons 5 \text{R} = \text{C}^- + 4 \text{Mn}^{2+} + 6 \text{H}_2\text{O} \quad \text{(S4.20)}
\end{align*}
\]

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Two Aldehydes

\[ 3 \text{C}=\text{C} + 4\text{MnO}_4^- + 4\text{H}^+ \rightleftharpoons 6\text{R}-\text{C}^-\text{O} + 4\text{MnO}_2(s) + 2\text{H}_2\text{O} \]  \hspace{1cm} (S4.22)

\[ \text{C}=\text{C} + \text{MnO}_4^- + \text{H}^+ \rightleftharpoons 2\text{R}-\text{C}^-\text{O} + \text{MnOOH}(s) \]  \hspace{1cm} (S4.23)

\[ 5 \text{C}=\text{C} + 4\text{MnO}_4^- + 12\text{H}^+ \rightleftharpoons 10\text{R}-\text{C}^-\text{O} + 4\text{Mn}^{2+} + 6\text{H}_2\text{O} \]  \hspace{1cm} (S4.24)

Diol

\[ 3 \text{C}=\text{C} + 2\text{MnO}_4^- + 2\text{H}^+ + 2\text{H}_2\text{O} \rightleftharpoons 3\text{R}-\text{C}^-\text{C}^-\text{R} + 2\text{MnO}_2(s) \]  \hspace{1cm} (S4.25)

\[ 2 \text{C}=\text{C} + \text{MnO}_4^- + \text{H}^+ + 2\text{H}_2\text{O} \rightleftharpoons 2\text{R}-\text{C}^-\text{C}^-\text{R} + \text{MnOOH}(s) \]  \hspace{1cm} (S4.26)

\[ 5 \text{C}=\text{C} + 2\text{MnO}_4^- + 12\text{H}^+ + 2\text{H}_2\text{O} \rightleftharpoons 5\text{R}-\text{C}^-\text{C}^-\text{R} + 2\text{Mn}^{2+} \]  \hspace{1cm} (S4.27)

4.6.2 3-Cyclopentene-1-carboxylic acid

A second model compound we employed was 3-cyclopentenecarboxylic acid (CPCA). Figure S4.1 show a proposed pathway for MnO$_4^-$ oxidation of CPCA. The fully oxidized product where the alkene double bond has been broken and oxidized to two carboxylic acid groups known as “tricarballylic acid” is commercially available. We report peak areas for the acid-aldehyde intermediate, and the diol product.
Methods for CPCA

All aqueous solutions were prepared from reagent grade chemicals and distilled, deionized water (Milli-Q water, 18 MΩ-cm resistivity, Millipore Corp., Milford, MA). All bottles and glassware were rinsed with distilled water, soaked in 5 M nitric acid overnight, rinsed with distilled water and Milli-Q water and then air-dried.

3-Cyclopentene-1-carboxylic acid and tricarballylic acid were purchased from Aldrich (St. Louis, MO). All other chemicals used were from the same suppliers mentioned in 4.2.1.

All experiments were performed in 100 mL polypropylene bottle reactors placed in a 25 ± 1°C constant temperature bath. Reaction solutions were continuously stirred with Teflon-coated stir bars and sparged with N₂(g). The N₂(g) sparge line is bubble through water before reaching the experimental solution to minimize evaporation of the solution. Before every experiment, fresh stock solutions of CPCA, KMnO₄, NaCl, and the buffer of interest were prepared in Milli-Q water. Solutions contained 5.0 mM acetate buffer to maintain pH at 4.25 and 5.0 mM carbonate buffer at pH 10.0. Sufficient stock of NaCl was added to adjust the ionic strength to 10 mM.

Samples were taken after 12 hours of contact time and filtered with 0.2 µm pore diameter track-etched polycarbonate filter membranes (Whatman, UK). The quenching solution was not used. LC/MS analysis was used to determine concentration of CPCA and tricarballylic acid oxidation product. Peak areas a peaks with m/z corresponding to diol product and acid-aldehyde intermediate were also measured. Concentration cannot be determined for diol product and acid-aldehyde because authentic standards were unavailable. HPLC separation was conducted using a Waters 2795 separation module (Milford, MA) with a 4.6 x 250 mm, 5 µm particle size, Atlantis T3 column (Waters, Milford, MA). HPLC eluent was
isocratic 85% 0.2% acetic acid, 15% methanol at a 1 mL min\(^{-1}\) flow rate. MS detection was conducted in the same manner as described for SDCA (4.2.5).

**Results and Discussion for CPCA**

The products generated from \(\text{MnO}_4^-\) oxidation of 3-cyclopentencarboxylic acid (CPCA) were starkly different at pH 4.25 than at pH 10.0 Figure S4.2. In both solutions, complete loss of CPCA was observed after 24 hours when \([\text{MnO}_4^-]_0\) was greater than 300 \(\mu\text{M}\). The diol product was detected at pH 10.0, but not at pH 4.25. The largest peak area for diol was observed at 400 \(\mu\text{M}\) \([\text{MnO}_4^-]_0\) and then decreased with increasing \([\text{MnO}_4^-]_0\). At pH 4.25, concentrations of tricarballylic acid were below 5 \(\mu\text{M}\) when \([\text{MnO}_4^-]_0\) was less than 500 \(\mu\text{M}\). Tricarballylic acid concentration increased to between 50 and 63 \(\mu\text{M}\) at permanganate concentrations between 1000 and 1800 \(\mu\text{M}\). At pH 10, tricarballylic acid increased slightly with increasing \([\text{MnO}_4^-]_0\), but did not exceed 7.2 \(\mu\text{M}\). Note that in control experiments in solutions of \(\text{MnO}_4^-\) and tricarballylic acid no loss of either compound was observed. At pH 4.25, the largest peak areas of acid/aldehyde arose when \([\text{MnO}_4^-]_0\) was in the range of 250 – 750 \(\mu\text{M}\), but it decreased at higher \([\text{MnO}_4^-]_0\). Only small peak areas of acid-aldehyde were detected at pH 10.0.

At pH 4.25, we expect that tricarballylic acid will be the fully oxidized product, and that acid/aldehyde would be an intermediate (Figure S4.1). Consistent with this, tricarballylic acid is favored at high \([\text{MnO}_4^-]_0\), and acid/aldehyde is favored at intermediate \([\text{MnO}_4^-]_0\) (Figure S4.2). However, even at the highest \([\text{MnO}_4^-]_0\) (1800 \(\mu\text{M}\)), only 62 \(\mu\text{M}\) tricarballylic acid has been generated out of 150 \(\mu\text{M}\) of CPCA initially added. Even though the \(\text{MnO}_4^-\) concentration was in greater than an order-of-magnitude excess, CPCA was not fully oxidized to tricarballylic acid within 24 hours. The dialdehyde intermediate, which we did not monitor, may account for some CPCA that was oxidized by \(\text{MnO}_4^-\), but not to
acid/aldehyde or tricarballylic acid. Because dialdehyde, acid/aldehyde, and tricarballylic acid are members of a series of oxidation reactions the amount generated after 24 hours will depend on the rate of the two aldehyde oxidation reactions ($k_4$ and $k_5$ on Figure S4.1). If $k_4$ is smaller than $k_5$ the concentration of dialdehyde will increase over time and the concentration of acid/aldehyde and tricarballylic acid will be limited. Unfortunately, we could not determine $k_4$ and $k_5$ directly, but they must be very slow because not all of CPCA is converted to tricarballylic acid in 24 hours despite the excess of MnO$_4^-$, Of course, CPCA may have been converted into a different product. Notably, we did not monitor the ketol. The ketol was a major product for SDCA (Figure 4.6), and may also be a major product for CPCA.

At pH 10.0, tricarballylic acid is not expected to be the major product: only 7.2 µM was generated at the highest [MnO$_4^-$]$_0$. Instead of acid/aldehyde and tricarballylic acid, the diol product was detected. High concentrations of OH$^-$ at pH 10.0 should favor the diol, which results from hydrolysis of the cyclic hypomanganate ester (Equation 4.9). The decrease in diol peak area after reaching a maximum at 400 µM [MnO$_4^-$]$_0$ could be due to higher oxidant dose favoring ketol, which is a more highly oxidized product than the diol (Table 4.1).
Table S4.1. Oxidizing equivalents (OE) measured after 30 minutes of contact time in solutions of 20 µM cis-stilbenedicarboxylic acid (SDCA) and MnO$_4^-$ (Figure 4.6). All OE reported in µEq L$^{-1}$.

<table>
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<tr>
<th>Buffer (pH)</th>
<th>[MnO$_4^-$]$_0$</th>
<th>Initial</th>
<th>Unfiltered</th>
<th>Filtered</th>
<th>Particulate</th>
<th>Transferred</th>
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<td>5.0 mM Acetate (pH 5.0)</td>
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Table S4.2. Concentrations and peak areas determined using LC/MS. Same conditions as Figure 4.6.

<table>
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<tr>
<th>Buffer (pH)</th>
<th>[MnO₄⁻]₀ (µM)</th>
<th>SDCA (µM)</th>
<th>Ald&lt;sup&gt;a&lt;/sup&gt; (µM)</th>
<th>Ac&lt;sup&gt;b&lt;/sup&gt; (µM)</th>
<th>K&lt;sup&gt;c&lt;/sup&gt; (Area)</th>
<th>Diol (Area)</th>
<th>Dione (Area)</th>
<th>B&lt;sup&gt;d&lt;/sup&gt; (Area)</th>
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<sup>a</sup> Aldehyde (4-carboxybenzaldehyde).  
<sup>b</sup> Acid (terephthalic acid).  
<sup>c</sup> Ketol.  
<sup>d</sup> Benzoic acid internal standard.
Table S4.3. Concentration of organic products and oxidizing equivalents (OE) after 30 minutes of contact time in solutions of 20 µM *cis*-stilbenedicarboxylic acid (SDCA) and MnO₄⁻ as shown on Figure 4.6. Concentrations are reported in µM and OE are in µEq L⁻¹

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<th>[MnO₄]₀</th>
<th>SDCA</th>
<th>[Ald]</th>
<th>[Ac]</th>
<th>[K]</th>
<th>[MB]</th>
<th>OE(t)</th>
<th>OE(p)</th>
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Table S4.4. Known pK\textsubscript{a} values for chemicals used in this chapter. Acid dissociation constants were corrected to zero ionic strength using the Davies equation (I), when necessary.

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<th>Chemical</th>
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<td>H\textsubscript{2}MnO\textsubscript{4} \rightleftharpoons MnO\textsubscript{4}\textsuperscript{−} + H\textsuperscript{+}</td>
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<td>2</td>
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\textsuperscript{a} in 50% aqueous ethanol at 25°C.
Figure S4.1. Proposed MnO₄⁻ oxidation pathway of 3-cylopentenecarboxylic acid. After the cyclic hypomanganate ester intermediate is formed, the pathway can diverge to aldehyde and acid products or a diol. Each compound is labeled with the molecular ion mass to charge ratio for which was used to detect each species.
Figure S4.2. 3-cyclopentencarboxylic acid (CPCA) consumed and products generated after 24 hrs. Conditions: 150 µM CPCA, 5 mM buffer, ionic strength adjusted to 10 mM.
Figure S4.3. Flow diagram for analysis of non-time course experiments using iodometric titration and LC/MS.
**Figure S4.4.** *cis*-Stilbenedicarboxylic acid (SDCA) loss in the absence of MnO$_4^-$ Ions. Ionic strength was adjusted to 10 mM by addition of NaCl.
Figure S4.5. Organic oxidation products of *cis*-stilbenedicarboxylic acid (SDCA) after 30 min of contact time (corresponds to Figure 4.6). Mass balance is calculated using Equation 4.13. Conditions: 20 µM SDCA, ionic strength adjusted to 10 mM.
References


Chapter 5

Conclusion

5.1 Summary of Findings

In this dissertation we have detailed our experimental results for two major topics. The first is nucleophilic addition reactions and the proelectrophile pathway. We monitored reactions of nucleophiles with \( p \)-benzoquinone and patulin electrophiles and hydroquinone proelectrophiles that become electrophilic following oxidation. These reactions deserve detailed study because they could be involved in bound residue formation in soils and sediments. The second topic is \( \text{MnO}_4^- \) oxidation of alkenes. We identified a model compound where all of the expected oxidation products can be detected by LC/MS. This conclusion chapter will provide a summary of our findings on both of these topics and then propose some future directions for each topic.

5.1.1 Nucleophilic Addition Reactions

Sorption and desorption processes are integral to environmental fate and transport of many contaminants after they are released into the environment. Irreversible binding, often involving formation of a covalent bond, removes certain contaminants from the water column
and renders them biologically unavailable. Irreversibly bound contaminants are sometimes called “bound residues.” Nucleophilic addition mechanisms, such as Michael addition, allow contaminants containing nucleophilic functional groups to form covalent bonds.

In Chapter 2, we sought to explore structure-reactivity relationships for Michael addition by monitoring loss of two model Michael acceptor electrophiles: \(p\)-benzoquinone and patulin. We monitored loss of Michael acceptor in the presence of a suite of added nucleophiles which were para-substituted anilines, 4-methylimidazole, and 4-methoxyphenol in MOPS buffered solution at pH 7.0. Our results are consistent with pH, nucleophilicity and electrophilicity being the key factors that control reaction rate between nucleophiles and Michael acceptors. We also confirmed that aromatic amines with electron-donating group substituents are stronger nucleophiles than those with electron-withdrawing substituents. Loss of \(p\)-benzoquinone is observed in the presence of aminopyralid, clopyralid, and chloramben herbicides may indicate that these more complex nucleophiles can also form bound residues. Substituted anilines reacted at least an order-of-magnitude faster with \(p\)-benzoquinone than with patulin, suggesting that 1,4-addition to \(p\)-benzoquinone is faster than 1,6-addition to patulin.

We note experimenters must take care to select buffers carefully when conducting experiments concerning nucleophile-electrophile reactions in aqueous media. In aqueous media, \(H_2O\) and \(OH^-\) are both capable of acting as nucleophiles, which we call “hydration. We saw absorbance increase in MOPS, MES, and DEPP buffered solutions of \(p\)-benzoquinone that we attribute to adduct formation. Maleate exhibited no concentration effect, suggesting that it does not react with \(p\)-benzoquinone and may be a more suitable buffer choice. However, under our experimental conditions in the presence of added nucleophiles the loss rate of \(p\)-benzoquinone and patulin was much faster than in the absence of added nucleophile.
In Chapter 3, we introduced the concept of “proelectrophiles” and reported on some simple model proelectrophile experiments. In particular, we are interested in highly electrophilic benzoquinones with electron-withdrawing group substituents. Unlike \( p \)-benzoquinone, which served as a model electrophile in Chapter 2, these substituted benzoquinones must be generated in situ by oxidation of a hydroquinone proelectrophile.

We expect one-to-one stoichiometry for pyrolusite (\( \text{MnO}_2 \)) oxidation of hydroquinone proelectrophiles because reduction of \( \text{MnO}_2 \) is a 2 electron process and so is oxidation of a hydroquinone proelectrophile to the corresponding benzoquinone. Similarly, the stoichiometry of nucleophilic addition of an added nucleophile to a benzoquinone electrophile should also be 1-to-1. To probe reaction stoichiometry we conducted a series experiments using acetylhydroquinone as the proelectrophile and 4-ethylaniline as the nucleophile where we monitored consumption of acetylhydroquinone, consumption of 4-ethylaniline, and generation of Mn\( ^{II} \). Increased generation of Mn\( ^{II} \) with increasing concentration of added nucleophile indicates that addition products become oxidized to second or third generation benzoquinones and increased consumption of nucleophile indicates that nucleophilic addition leading the adducts (\( X–\text{Nu} \)) is favored at the expense of hydration. We also considered initial rates of consumption of acetylhydroquinone, consumption of 4-ethylaniline, and generation of Mn\( ^{II} \). All three initial rates increased with increasing concentration of proelectrophile. However, only the initial rate of consumption of nucleophile increased with increased concentration of nucleophile. The rate and extent of adduct formation depends on both the concentrations of proelectrophile and nucleophile but also on the rate constants for oxidation and addition. Lastly, we reported LC/MS evidence for monoadducts resulting from experiments with gentisic acid as the proelectrophile and nine different nucleophiles.

Our results expand the mechanistic understanding of the interplay between addition reactions and oxidation reactions. This may represent a significant sink for nucleophilic
contaminants and natural products in environments where Mn^{III,IV} (hydr)oxides oxidize organic matter such as at oxic/anoxic interface in sediments (1) or in manganese containing soils (2).

### 5.1.2 Permanganate oxidation of Alkenes

Technologies involving chemical oxidation are invaluable in water/wastewater treatment and subsurface remediation situations. In particular, oxidation by permanganate (MnO$_4^-$) can be employed to treat taste and odor problems, remove Mn$^{II}$ and Fe$^{II}$, and remove precursors for disinfection byproducts among other uses.

MnO$_4^-$ can oxidize a variety of inorganic chemicals and functional groups within organic molecules. A simple and common MnO$_4^-$ oxidizable functional group is the alkene carbon-carbon double (−C=−C−). Oxidation is thought to be initiated by MnO$_4^-$ attack on the double bond leading to a cyclic Mn$^V$ intermediate. The ultimate products result from competition of hydrolysis and oxidation reactions the following formation of an intermediate, which result in distribution of products is generated that depends on the pH and the dose of MnO$_4^-$.

Based on a literature review, the products of MnO$_4^-$ oxidation of alkenes are expected to contain aldehydes, carboxylic acids, ketols, diones, and diol functional groups. However, most existing reports focus on a subset of these products. We monitored a more complete distribution of oxidation products by employing cis-stilbenedicarboxylic acid and 3-cyclopentenecarboxylic acid as convenient model alkenes, which could be detected even without authentic standards for some of the products. cis-Stilbenedicarboxylic acid was oxidized primarily to a ketol product or broken into two aldehyde products after 30 minutes of contact time and 10 times excess of permanganate. We also observed oxidation of the ketol to a dione and the aldehyde to a carboxylic acid; however this reaction is much slower.
We also used filtration and iodometric titration to attribute oxidizing equivalents remaining after 30 min to Mn\textsuperscript{III,IV} solids and residual MnO\textsubscript{4}. Identification of useful model compounds such as cis-stilbenedicarboxylic acid and 3-cyclopentenecarboxylic acid (in the Supporting Information for Chapter 4) enhances our ability to observe effects of medium composition and experimental conditions for MnO\textsubscript{4} oxidation of alkenes in the laboratory.

### 5.2 Future Research

Our results reported here would benefit from additional experimental data and some suggestions are given to future experimenters. Hopefully, they will spark further research into nucleophilic addition reactions in the environment and oxidation by MnO\textsubscript{4} that will lead to useful tools for the environmental chemistry community.

#### 5.2.1 Nucleophilic Addition and the Proelectrophile Pathway

The ratio plots (Figure 3.4) are an informative tool for discussing competition between hydration and nucleophilic addition for reaction with the generated benzoquinone electrophile and similar data should be taken for proelectrophiles with other sidegroups, for example hydroquinone or methylhydroquinone. Similar experiments with two nucleophiles from the same family, e.g. 4-nitroaniline and 4-methylaniline, may yield more detailed information about the influence of nucleophilicity and basicity. Blocking surface sites by including phosphate or pyrophosphate could help determine which steps of the proelectrophile pathway take place at the surface or are surface limited.

Pseudo-first-order kinetic studies monitoring loss of \(\rho\)-benzoquinone could be used as a simple predictor for rate of bound residue formation. Colón et al (3) correlated pK\textsubscript{a},
Hammett $\sigma$ constant, and half-wave potential with loss rate of aromatic amines in soil samples. While, these molecular descriptors worked well for aromatic amines, they are often not available for complicated compounds like many organic contaminants. Pseudo-first-order rate constant for loss of $p$-benzoquinone could also correlate with loss rate to soils and sediments and can be determined by simple, inexpensive laboratory experiments. Loss of $p$-benzoquinone experiments are much cheaper to perform and containing fewer confounding factors than experiments in the field or experiments using whole soil or sediment samples. Potential nucleophiles could be screened for its rate of reaction with $p$-benzoquinone and the rate would be normalized by the rate of $p$-benzoquinone loss of a “benchmark nucleophile resulting in one weighting factor per nucleophile. A similar approach was taken by U.S. EPA (4) when developing toxic weighting factors (TWF) which allow wastewater discharges to be directly compared to each other in terms of toxic-weighted pounds equivalent even when the particular pollutants that the wastewater contains are different. Using a complex dataset of human health criteria and chronic aquatic life toxicity data, EPA developed factors relative to copper where pollutants with TWF greater than copper are more toxic. The weighting factors developed from rate of loss of $p$-benzoquinone would rank the nucleophiles in terms of likelihood to form covalently-bound residues. It could be a basis for selecting “green chemicals when performing an alternatives analysis for chemicals currently being used in industry and commerce. Chemicals that are quickly removed from the water column via binding to organic matter might be preferable to chemicals that do not bind and might persist longer the environment. If necessary, rates for loss of nucleophiles with known weighting factors in a particular location could be estimated by conducting a single whole soil or sediment experiment using the “benchmark nucleophile and a representative soil or sediment sample. Then, the amount of contaminants lost due to irreversible binding could
be estimated in fate and transport models of compounds containing a nucleophilic functional group.

5.2.2 Oxidation by Permanganate

Our experiments were continuously sparged with N₂ to minimize the concentration of O₂ in solution. In water treatment, the source water may contain higher concentrations of O₂ which could oxidize intermediates or products involved in MnO₄⁻ oxidation. This could change the distribution of the products or lead to new products.

The influence of natural organic matter (NOM) on MnO₄⁻ oxidation of alkenes should be evaluated, as well as the effect of oxidation by MnO₄⁻ on the properties and structure of NOM itself. The chemical structure of NOM is complicated and difficult to characterize. Redox properties of NOM samples depend on sampling location, because of differing environmental conditions that lead to NOM formation. Aeschbacher et al. (5) reported that electron accepting capacity of humic substance samples was linearly correlated with C/H elemental ratio and aromaticity estimated from ¹³C NMR spectra. Perhaps a similar correlation exists for MnO₄⁻ oxidation rate and pathway because MnO₄⁻ oxidation is selective to certain functional groups. Based on chemical structure, some NOM samples may be more readily oxidized by MnO₄⁻ than others. Similarly, MnO₄⁻ may be less effective at promoting aggregation or removing disinfection byproduct precursors depending on the NOM sample origin. Humic acids may also differ from fulvic acids in terms of MnO₄⁻ oxidation reactivity. Aeschbacher et al. (5) also reported that electron accepting capacity was highest for terrestrial humic acids and lowest for aquatic fulvic acids.
References


Vita

Phillip Martin Flanders was born in St. Louis Park, Minnesota on July 8, 1985. He grew up in Chanhassen, Minnesota and graduated from Chaska High School in Chaska, Minnesota in 2003. He received a B.S. with a double major in Chemical Engineering and Chemistry with a minor in Japanese Language and Culture in 2007 from Rose-Hulman Institute of Technology (Terre Haute, Indiana). His undergraduate research work at Rose-Hulman focusing on reaction pathways of TiO$_2$ photodegradation of sulfamethoxazole in 2004. He began graduate studies in 2007 at Johns Hopkins University (Baltimore, Maryland) in August of 2007, earning the M.S.E. in Geography and Environmental Engineering in 2009. His research at Johns Hopkins focused on highly electrophilic benzoquinone Michael acceptors generated via MnO$_2$ oxidation, and the product distribution resulting from permanganate oxidation of alkenes. Upon completion of his graduate studies, Phillip accepted a position as an Environmental Engineer with the U.S. Environmental Protection Agency in the Engineering and Analysis Division of the Office of Water located in Washington, D.C.