INVESTIGATING THE STABILITY OF AQUEOUS MN(III) IN THE PRESENCE OF HUMIC SUBSTANCES AND MEASURING MANGANESE SPECIATION WITH ALPHA, BETA, GAMMA, DELTA-TETRAKIS (4-CARBOXYPHENYL)PORPHINE

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A thesis submitted to the Faculty and Board of Trustees of the Colorado School of Mines in partial fulfillment of the requirements for the degree of Master of Science (Geochemistry).

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ABSTRACT

Manganese is a significant component of natural waters, and is found as Mn(II), (III), and (IV). Mn(III) had long been considered too unstable to be a dissolved species in natural waters, and ligand stabilization is required to prevent it from disproportionating. However, Mn(III) is present under certain conditions in ocean waters. Our work addressed the ability of humic substances to stabilize Mn(III). Mn(II) and (III) concentrations were measured by the modeling of complexation kinetics of both manganese species with the porphyrin ligand α,β,γ,δ-tetrakis(4-carboxyphenyl)porphine (TCPP) to form Mn(III)-TCPP. We found that dissolved Mn(III) was not stabilized in solution by humic substances and that, instead, a colloidal manganese species formed that was reactive with TCPP. In addition, we found that high concentrations of pyrophosphate and citrate interfere with the TCPP method by binding to cadmium, which is required to catalyze the reaction between TCPP and Mn. This was found to slow the rates of complexation of Mn(II) and (III).
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ACKNOWLEDGEMENTS

Many, many thanks go to my thesis advisor, Dr. Tina Voelker. Thanks also to my committee members Dr. Shubham Vyas and Dr. John Spear. For their help with instrumentation and measurements, I would like to thank Ramona Figueroa, Jim Ranville, Daniel Van Hoomissen, and Megan Otting.

I would also like to thank the Department of Chemistry & Geochemistry for the funding I received through the Teaching Assistant program.
CHAPTER 1
INTRODUCTION

Traditionally, studies on manganese chemistry in the environment have focused on the two most common oxidation states of manganese: Mn(II) and Mn(IV). In the aqueous state, manganese is often Mn$^{2+}$, while solid manganese is typically Mn(IV) or Mn(III,IV) oxides. The redox chemistry of manganese itself is controlled by various other species in an aqueous system, including pH, metal content, and dissolved oxygen. In highly oxygenated waters, Mn(III,IV) oxyhydroxides are common, while Mn(II) dominates in reducing environments (Madison et. al., 2013, Tipping et al., 1984). Both of these species are important in natural aquatic systems, playing a role in redox cycling and metal transport (Laxen et al., 1984; Davison, 1992). The cycling between manganese oxides and Mn(II) governs the mobility and bioavailability of trace metals, as sorption of metals to colloidal manganese oxides can enhance metal transport. Mn(II) can also act as an electron donor for microbes (Tipping et al., 1984), while manganese oxides have been shown to oxidize a wide range of organic compounds, including many pollutants, as well as act as an electron acceptor for microbes (Remucal & Ginder-Vogel, 2014). Because these two species have a broad effect on aqueous chemistry in natural waters, there has been considerable interest in also investigating the role aqueous Mn(III) may play, as it can readily form Mn(II) or Mn(IV), and play a similar environmental role as either species by donating or accepting electrons from microbes or other chemical species.

Aqueous (dissolved) Mn(III) has long been theorized to be an important part of aqueous redox chemistry as either an electron donor or acceptor (Kostka et al., 1995), but because Mn(III) was thought to rapidly disproportionate, it has traditionally been ignored in studies of manganese in the environment. However, the high reactivity of Mn(III) with other dissolved species means that it may act in a variety of ways in a system, either reducing or oxidizing organic matter and metals as the predominating chemical conditions allow. Work by Parker et al. (2004) showed that Mn(III) is readily stabilized by a siderophore produced by the Mn(II)-oxidizing bacterium Pseudomonas putida. This suggests that Mn(III) plays an important role in the metabolism of that and other microbes. Mn(III) has also been shown to degrade a variety of pollutants, including bisphenol A, faster than permanganate when a stabilizing ligand is present (with the exception of the ligand EDTA, whose presence did not increase the rate of pollutant oxidation relative to Mn(III) in the absence of ligands) (Jiang et al., 2010; Jiang et al., 2012; Roderick et
al., 2013). As a result, the stabilization of Mn(III) formed by the reduction of permanganate by ligands increased the effectiveness of oxidation treatments with permanganate. In addition, Mn(III) stabilized by the ligand pyrophosphate (PP) was shown to oxidatively dissolve uranium (IV) oxide in an anoxic solution more rapidly than did dissolved oxygen (Wang et al., 2014).

Because aqueous Mn(III) is short-lived unless stabilized by ligands, investigating the presence of Mn(III) in water samples has been difficult. Only recently have studies tried to measure Mn(III) in natural waters. Trouwborst et al. (2006) were able to detect Mn(III) in complexes with cathodic stripping voltammetry, while Lin et al. (2012) detected Mn(III) in incubations of Mn(IV)-reducing bacteria and Webb et al. (2005) found manganese(III) was a product of Mn(II) oxidation by bacteria. A kinetic method to make accurate measurements of both Mn(III) and Mn(II) at the low concentrations expected in most natural conditions was developed recently by Madison et al. (2011). Their method relies on measuring how quickly Mn(II) and Mn(III) react to form the Mn(III) complex with α,β,γ,δ-tetrakis(4-carboxyphenyl)porphine (TCPP). Mn(II) will complex with TCPP quickly, then rapidly oxidize to Mn(III) to form Mn(III)-TCPP. Mn(III) complexes directly with TCPP, but does so much more slowly than Mn(II). The rate of Mn(III)-TCPP formation can be measured spectrophotometrically, and the rate of formation can be modeled as the sum of the rate of reaction of the two manganese species. This modeling then yields the initial concentration of Mn(II) and Mn(III).

This method has since been applied in investigating Mn(III) concentrations in the anoxic pore waters of sediment cores taken from the St. Lawrence Estuary (Madison et al., 2011; Madison et al., 2013; Oldham et al., 2015). Their research showed Mn(III) could account for nearly all dissolved manganese at depth and in pore waters, likely as a result of the oxidation of Mn(II) as it diffused upwards. The work by Oldham et al. suggested that Mn(III) was being stabilized by both strongly and weakly binding natural ligands, which resulted in high concentrations (~ 7 µM) of dissolved Mn(III). However, their method of investigating Mn(III) in natural waters has not, to our knowledge, been used to examine surface waters. This represents a significant gap in the understanding of the role manganese species play in natural redox chemistry.

It has long been known that Mn(III) can be stabilized by ligands such as pyrophosphate, EDTA and citrate (Klewicki & Morgan, 1998), but the capacity of natural organic matter (NOM)
to stabilize Mn(III) has not been successfully investigated. The research detailed below was
designed to assess the ability of NOM to stabilize dissolved Mn(III), and to investigate other
possible interactions (e.g. redox reactions) between Mn(III) and NOM. Suwannee River Fulvic
Acid (SRFA) was chosen as an analogue for NOM in a natural environment. SRFA, like most
NOM, has functional groups that act as chelating groups for a variety of metals. We
hypothesized that these groups would chelate with Mn(III) as Mn(III) was produced in solution.
We used the kinetic TCPP method of Madison et al. (2011) to examine changes in manganese
speciation as a function of time in our experimental solutions. We also examined the effect of
high concentrations of the ligands pyrophosphate and citrate on the TCPP method, as some of
our planned experiments relied on high ligand concentrations.
CHAPTER 2
METHODS

In this chapter the experimental procedures used in our measurements are outlined. They include an outline of the TCPP measurement techniques of Madison et al. (2011), but with the method refinements we have made. Section 2.1 is a list of stock solutions used, with instructions on their preparation.

2.1 - Reagent Solutions

*Acetate buffer* - 25 mL of glacial acetic acid was diluted into 100 mL of milli-Q water and adjusted to a pH of 4.00 with drop-wise additions of 1 M sodium hydroxide (and 1 M hydrochloric acid if needed).

*Cadmium(II) chloride* - A cadmium(II) chloride solution was prepared by dissolving 0.022 g of cadmium chloride n-hydrate in 100 mL of milli-Q water. As cadmium chloride is extremely hygroscopic, the concentration of cadmium was verified with inductively-coupled plasma atomic emission spectrometry (ICP-AES) (see section 2.2). The measured cadmium concentration in our stock solution was 0.61 mM.

*N,N Diethyl-1-4-phenylenediamine sulfate (DPD) Stock* – An acidified 6.1 mM stock solution of DPD was prepared by dissolving 0.010 g of N,N Diethyl-1-4-phenylenediamene sulfate (97%, Sigma Aldrich) and 0.80 mL of 7 M nitric acid in 100 mL of milli-Q water.

*DPD Reagents* - 12.5 mL of sodium bicarbonate solution were added to a small beaker. 0.5 mL of the DPD stock was added next, followed by 1.0 mL of the sodium phosphate buffer. This solution was mixed and allowed to react for 5-10 minutes before use.

*4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer* - A 0.5 M HEPES buffer was prepared by dissolving 2.98 g of HEPES in 25 mL of milli-Q water, and adjusting the pH to 7.0 with 1 M hydrochloric acid and 1 M sodium hydroxide.
**Imidazole buffer** - 50 mL of 0.025 M sodium tetraborate was mixed with 25 mL of imidazole stock and 20 mL of 0.1 M hydrochloric acid. The pH was adjusted to 8.00 with 1.0 M hydrochloric acid.

**Imidazole stock** - A 0.6 M stock solution of imidazole was prepared from 4.068 g of newly-purchased imidazole (Sigma Aldrich) dissolved in 100 mL of milli-Q water.

**Manganese(III) Pyrophosphate** - Manganese(III) acetate (Sigma Aldrich) and sodium pyrophosphate (Sigma Aldrich) were used to make a 100 mL stock solution. 0.0122 g of manganese(III) acetate were dissolved in 25 mL methanol, to ensure full dissolution. 0.122 g sodium pyrophosphate was added, and the solution was topped up to 100 mL with milli-Q water. The pH was adjusted to 7.0 with 1.0 M sodium hydroxide.

**Manganese(II) Chloride** - A 2.3 mM stock solution of manganese(II) chloride was prepared by measuring 0.0474 g of manganese(II) chloride tetrahydrate salt (Sigma Aldrich) into a 100 mL volumetric flask, acidifying by drop-wise addition of 3 M hydrochloric acid to pH 3.0 as measured by pH probe.

**Potassium permanganate stock** - 1.975 g of potassium permanganate was dissolved in 100 mL of milli-Q to yield a 0.1250 M solution, and stored in the dark.

**Sodium bicarbonate solution** - 0.023 g of sodium bicarbonate was dissolved in 250 mL of milli-Q water to yield a 1.0 mM solution.

**Sodium phosphate buffer** - A 0.5 M sodium pyrophosphate buffer was prepared by dissolving 5.24 g of monosodium phosphate and 2.14 g of disodium phosphate in 100 mL of milli-Q water, and adjusting the pH to 6.0 (as measured by pH probe) with 1 M hydrochloric acid and 1 M sodium hydroxide.

**Sodium tetraborate solution** - A 0.025 M solution of sodium tetraborate was prepared by dissolving 4.769 g in 500 mL of milli-Q water.
Suwannee River Fulvic Acid (SRFA) stock - A 320 mg/l stock solution of SRFA was prepared in 25 mL volumetric flasks using freeze-dried SRFA and milli-Q water. The stock solution was stored at 4°C in the dark for no longer than 2 months.

TCPP - 500 mL of TCPP stock was prepared by dissolving 0.072 g of TCPP in 0.5 mL of 1 M NaOH, and 4.5 mL milli-Q water. Once the TCPP was dissolved, the solution was topped up to a total volume of 500 ml. It was stored in an opaque bottle at 4°C.

2.2 - Verification of Solution Concentrations

Total manganese concentrations of the permanganate stock solution and the experimental solutions were verified with atomic absorption spectroscopy. Five standard manganese solutions of 0, 1.0, 2.5, 5.0, and 10.0 µM were prepared from a 10,000 ppm manganese standard, concentrated hydrochloric acid, and milli-Q water. Solutions to be analyzed were diluted to about 5 µM total manganese, and hydrochloric acid was added to achieve a 1% solution of acid. The permanganate stock, and Mn(III)/SRFA solutions were consistent with expected manganese concentrations when tested with the AA. The concentration of Mn(II) in the manganese(II) chloride stock solution was determined using the TCPP method (outlined in section 2.3).

Cadmium concentrations were determined by submitting an unaltered sample of the stock for analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES). Though the concentration of cadmium should have been 1.2 mM given the mass weighed, the salt is extremely hygroscopic, which resulted in a smaller concentration. To handle this, the cadmium concentration was measured with ICP-AES, and the volumes of the cadmium solution added to the reagent solution were increased to yield the correct final concentration of cadmium.

2.3 - Kinetic Determination of Mn(II) and (III)

The examination of Mn(II) and (III) concentrations was conducted using a modified version of the TCPP method outlined in Madison et al. (2011). Briefly, the method relies on measuring the rate of complexation of manganese with TCPP spectrophotometrically. Mn(II) will complex quickly with TCPP, and once complexed it will oxidize to Mn(III). This Mn(III)-TCPP complex absorbs at 468 nm. Mn(III) will directly complex with TCPP much more slowly.
By measuring the rate at which the 468 nm absorbance peak grows and fitting the data as the sum of two first-order reactions, the concentrations of Mn(II) and (III) can be determined (Ishii et al., 1982; Madison et al., 2011).

The reagents for the kinetic TCPP method are mixed in the following ratios: 360 μL of TCPP stock, 120 μL of imidazole buffer, 120 μL of cadmium stock, and 2.3 mL of milli-Q water. This results in 2.90 mL of solution, which is enough for one TCPP kinetic run. If more runs are desired on the same day, the volume of reagents is simply scaled up to meet the required number. This reagent solution does not appear to be stable overnight, as the solution changes color and a gray precipitate develops. In this study, it was used within four hours of mixing or less, which gave consistent baseline values for our experiments. Solutions of manganese(II) chloride and manganese(III) pyrophosphate were also prepared in order to reproduce the laboratory work of Madison et al. (2011). Manganese(II) chloride was prepared as described in Madison et al. (2011), but the manganese(III) pyrophosphate was prepared by first dissolving manganese(III)-acetate in methanol to ensure full dissolution, and then mixing into a deoxygenated solution of pyrophosphate, which differed from the preparation described in Madison et al. (2011) (see section 2.1).

The TCPP-Cd(II) reagent mixture was mixed in large quantities (typically enough for eight kinetic runs), and stored in a light-proof container at room temperature. A Thermo Evolution 300 spectrophotometer was used to collect kinetic data. Because ambient light will cause the Cd(II)-TCPP peak at 432 nm to broaden, the absorbance at 468 nm drifts higher under illuminated conditions. This can make determining a consistent value for reagent absorbance baselines at 468 nm difficult, so it is important to use a lightproof instrument to measure the absorbance of the TCPP mixture. The Thermo Evolution 300 spectrophotometer (1) uses a monochromator which allows only one wavelength of light through the sample during kinetic measurements and (2) relies on a xenon lamp, which uses very short flashes of light to measure absorbance, minimizing any drift in the reagent baseline over very long kinetic experiments. Mn contamination of the cuvette was also found to cause baseline drift. To eliminate this, cuvettes were cleaned by first soaking in a 10 mM hydroxylamine solution at pH ~3 overnight, then a 1.0 M oxalic acid bath overnight. The cuvettes were rinsed with milli-Q water before use.
Figure 2.1 shows a typical reagent baseline plot with a typical manganese kinetic run. There was little baseline drift for the duration of the experiment, with the baseline increasing by 0.006 absorbance units per hour due to light exposure in the spectrophotometer. This absorbance increase corresponds to an ~70 nM increase in the measured manganese per hour, which is just below the 100 nM detection limit for this method reported by Madison et al. (2011).

After the baseline absorbance of the TCPP reagent mixture was determined, the manganese sample (typically 100 µL) was pipetted into a clean 1-cm glass cuvette. The TCPP-Cd(II) reagent mixture was then quickly pipetted on top of the manganese sample, bringing the total cuvette volume to 3.00 ml, and kinetic measuring of the sample began. Adding the TCPP-Cd(II) reagent on top of the manganese sample allows for rapid mixing of the reagents (as the volume is 29 times larger), and as long as the cuvette is already in the spectrophotometer, absorbance measurements can be made within 5 seconds of mixing. The time elapsed between mixing the reagent and manganese solutions and the first absorbance measurement is also noted on a stopwatch. During measurements, the spectrophotometer was set to measure absorbance at 468 nm either every 5 seconds for a total of ten minutes, which allows for ready capture of data.
related to the formation of the TCPP-Mn(III) complex, and matches the experimental procedure of Madison et al. (2011). For a given set of experiments, the measured TCPP baselines  

![Graph](image)

Figure 2.2 – Raw absorbance data (green), the model fit for the data (gray), and the individual modeled kinetic curves for Mn(II) (blue) and the slow manganese species (red) for the unfiltered data point at 98.4 hr (Table 3.2). The concentration of Mn$^{2+}$ was measured as 75.3 µM (k$_{Mn^{2+}}$: 0.0906 sec$^{-1}$) while Mn$_{Slow}$ was measured as 9.0 µM (k$_{Mn_{Slow}}$: 0.0044 sec$^{-1}$). The absorbance baseline for this experiment was about 0.040.

(collected before each run) were consistent with each other, so long as the measurements are conducted within four hours of the initial reagent baseline measurement, and the TCPP reagent solution was kept in the dark. Before the data can be modeled, the data must be adjusted to account for the lag time between mixing the reagent solution with the manganese solutions and the first measured absorbance point. To do so, the times of each measurement are adjusted upward by the lag time. A time point at zero seconds is then added to the data, and the absorbance for this point is set to the measured TCPP reagent baseline.

The kinetic data are then worked up using the same principle as Madison et al. (2011). The formation of the Mn(III)-TCPP complex is modeled as being the result of two first-order reactions occurring at different rates. Using a custom Mathematica code (see Appendix), the measured absorbance values (Abs) at a given time ($t$) were fit to Equation 1 using a least-squared difference solver and four fitting parameters: the absorbance corresponding to the initial
concentration of Mn(II) \( (A_{Mn(II)}) \) and a slow manganese species \( (A_{Mn_{slow}}) \) in the cuvette, as well as the reaction rate coefficients for both Mn(II) \( (k_{Mn(II)}) \) and the slow manganese species \( (k_{Mn_{slow}}) \):

\[
Abs = A_{Mn(II)} \left( 1 - e^{-k_{Mn(II)}t} \right) + A_{Mn_{slow}} \left( 1 - e^{-k_{Mn_{slow}}t} \right) + \text{baseline} \quad (Eqn.1)
\]

Note that at \( t=\infty \), \( Abs \) approaches the sum of \( A_{Mn(II)}, A_{Mn_{slow}}, \) and \( \text{baseline} \). The baseline absorbance of the TCPP reagent mixture is read from the \( t=0 \) data point and included in the model. Once the model has fit the data, \( A_{Mn(II)} \) and \( A_{Mn_{slow}} \) are divided by the molar absorptivity for Mn(III)-TCPP \( (95,400 \, M^{-1} \, cm^{-1}) \) to yield the initial concentration of each species in the cuvette, \( [Mn(II)]_0 \) and \( [Mn_{slow}]_0 \) (Madison et al., 2011). These values only represent the concentration in the cuvette; to yield concentrations in the Mn(III)-SRFA experimental solution they must be multiplied by 30, which is the dilution factor. Figure 2.2 shows an example of the graphical output of the model, with raw data and the model fit.
2.4 - Mn(III) Experiments

To examine the ability of SRFA to stabilize Mn(III), high concentrations of both SRFA and Mn were maintained in solutions. For most experiments, 100 µM Mn(III) was synthesized in 100 mL of a 10 mg/L SRFA solution using the reaction between Mn(II) and permanganate:

\[ 8H^+ + 4Mn^{2+} + MnO_4^- \rightarrow 5Mn^{3+} + 4H_2O \]

The SRFA solution was first prepared by mixing 2.00 mL of the pH 7.00 HEPES buffer and 3.125 mL of SRFA stock in a volumetric flask. When measurements of manganese concentrations and speciation were ready to begin, 4.00 mL of the stock Mn(II) solution was added, and the volumetric flask was filled nearly to the mark with milli-Q water. To initiate the reaction, 160 µL of 0.125 M potassium permanganate was added to the volumetric flask, the solution was topped off to the mark, and mixed by inverting. The reaction between permanganate and Mn(II) is faster (see Figure 2.3) than the reaction between the permanganate and SRFA, so we expect that most of the permanganate reacted with Mn(II) to form Mn(III), though some direct reduction of permanganate by SRFA probably also occurred, which could have produced manganese oxide, Mn(III) and/or Mn(II). Mn(III) solutions without SRFA were prepared as above, but omitting the SRFA.

Concentrations of Mn(II) and the slow manganese species were then determined at various times after making the Mn(III)/SRFA solution using the TCPP method described in section 2.3. Several time scales were examined in three separate experiments: from 1 to 30 minutes after the addition of permanganate into the reagent solution, and from 40 to 120 min after the permanganate addition, and from 1 day to 2 months after the permanganate addition. Each TCPP kinetic run was 10 minutes in length, in agreement with the procedure of Madison et al. (2011). For each experiment, the starting time of the TCPP run was considered the time of measurement, so a run begun one minute after the formation of the manganese/SRFA solution was considered a measurement of the speciation in the manganese/SRFA solution one minute after its formation.

2.5 - Analysis of Oxidation State of Colloidal Manganese

The DPD method described in Johnson and Chiswell (1993) was used to analyze the oxidation state of manganese in the colloidal material, presumed to be manganese oxide, observed in Mn(III)/SRFA experimental solutions. To measure the colloidal material’s oxidation
state, a known amount of material has to be trapped on a filter and reacted with DPD to dissolve the colloids. 5.0 mL of the Mn(III)/SRFA experimental solution was filtered with 0.02 µm Anotop filters. These filters were cleaned with 2-3 mL of 1.0 M hydrochloric acid and rinsed with 10-15 mL of milli-Q water before the experimental solution was filtered. Once the colloidal manganese was collected on the filter, the filter was rinsed with milli-Q water to remove any non-colloidal manganese or SRFA.

Mn(III,IV) oxides are mixtures of Mn(III) oxide (Mn₂O₃ or MnOOH) and Mn(IV) oxide (MnO₂). As such, the total moles of manganese oxide, mol_{MnOₓ}, are equal to the sum of the moles of Mn(III) and Mn(IV) oxide (mol_{MnO₃} and mol_{MnO₂}):

\[ mol_{MnOₓ} = mol_{MnO₃} + mol_{MnO₂} \quad (Eqn. 2) \]

Manganese oxides are strong oxidizing agents, and their ability to oxidize other species can be described in terms of oxidizing equivalents (OE). The total oxidizing equivalents in Mn(III,IV) oxides can be determined as follows, assuming the manganese is fully reduced to Mn(II):

\[ OE = Mol_{MnO₃} + 2 (Mol_{MnO₂}) \quad (Eqn. 3) \]

In order to measure the average oxidation state of the manganese oxides, DPD is reacted with manganese oxide colloids isolated from the solution on a 0.02 µm filter. The colorless DPD is oxidized by the colloidal manganese to a light pink species (known as Wurster’s dye) in a one-electron transfer. Mn(IV) has the potential to oxidize two molecules of DPD, while Mn(III) can only oxidize one DPD molecule. The molar absorptivity of Wurster’s dye (ε_{WD}) is given in Johnson and Chiswell (1993) as 9850 (mol/L)⁻¹·cm⁻¹ at 550 nm.

The total moles of colloidal manganese (mol_{MnOₓ}) collected by filtration can be determined by looking at the difference in total manganese concentration (as measured by AA) of a known volume (V_{Mn}) of manganese solution before ([Mn_i]) and after ([Mn_f]) filtration with a 0.02 µm Anotop filter:

\[ mol_{MnOₓ} = V_{Mn} [Mn_i] - V_{Mn} [Mn_f] \quad (Eqn. 4) \]

Passing a known volume of DPD solution (V_{DPD}) slowly through the filter allows the colloidal manganese to oxidize DPD to Wurster’’ dye. The spectrophotometrically determined concentration of Wurster’s dye [WD] allows for the measure of moles of oxidizing equivalents in the manganese colloid on the filter as follows:

\[ Mol_{OE} = V_{DPD} * [WD] \quad (Eqn. 5) \]
With the moles of oxidizing equivalent and of manganese colloid, the moles of Mn(III) and Mn(IV) can then be calculated:

\[
2mol_{MnO_x} - mol_{OE} = mol_{Mn(III)} \quad (Eqn. 6)
\]

\[
mol_{MnO_x} - mol_{Mn(III)} = mol_{Mn(IV)} \quad (Eqn. 7)
\]

2.6 - Effects of Cadmium to Ligand Ratios on the TCPP Kinetic Method

The kinetic TCPP method developed by Madison et al. (2011) relies on the exchange of Cd(II) complexed with TCPP for manganese. Without cadmium in the TCPP reagent solution, the rates of complexation with TCPP for either Mn(II) or Mn(III) become too slow to measure or distinguish from one another. Because Mn(III) requires high concentrations of ligands to be stabilized, there is the possibility that cadmium could be stripped from the TCPP by the ligands, which would limit the usefulness of the method. As some of our initially planned experiments called for high concentrations of citrate or pyrophosphate, we examined the effect that higher concentrations of citrate and pyrophosphate had on the Cd(II)-TCPP complex and on the kinetics of formation of the Mn(III)-TCPP complex.

The TCPP was diluted by a factor of 2 (to a concentration of 12 μM), so that the absorbance peaks for both Cd(II)-TCPP and TCPP could be measured on the spectrophotometer. The imidazole buffer was not diluted, as imidazole catalyzes the exchange of cadmium for manganese (Madison et al., 2011), and the cadmium concentration in the cuvette was either the full concentration required to stoichiometrically react with the TCPP (12 μM) or half the full concentration required (6 μM). In order to measure the actual concentration of the Cd(II)-TCPP complex spectrophotometrically, the molar absorptivities of both Cd(II)-TCPP at its absorbance maximum at 432 nm ($\epsilon_{Cd-TCPP}$), as well as the absorbance of TCPP at 432 nm ($\epsilon_{TCPP}$) must be known. $\epsilon_{Cd-TCPP}$ is given in Kilian and Pyrzyńska (2003) as 3.8×10⁵ M⁻¹·cm⁻¹, while $\epsilon_{TCPP}$ was determined to be 2.1×10⁴ M⁻¹·cm⁻¹ by a calibration plot of the absorbance at 432 nm as a function of TCPP concentration in the absence of cadmium (Figure 2.4). The concentration of the Cd(II)-TCPP complex ([Cd – TCPP]) was determined from the measured absorbance at 432 nm ($Abs_{432}$), given that the initial concentration of TCPP in the solution ([TCPP]₀) is known. Abs is given by:

\[
Abs_{432} = \epsilon_{TCPP} ([TCPP]₀ – [Cd – TCPP]) + \epsilon_{Cd-TCPP} ([Cd – TCPP]) \quad (Eqn. 8)
\]
This equation can then be solved for the concentration of the Cd(II)-TCPP complex concentration in the cuvette:

\[ [Cd - TCPP] = \frac{Abs_{432} - \varepsilon_{TCPP}[TCPP]_0}{\varepsilon_{Cd-TCPP} - \varepsilon_{TCPP}} \quad (Eqn. 9) \]

Determining the concentration of Cd(II)-TCPP spectrophotometrically allows us to examine the effect that strong ligands have on the complex. This is done by first measuring the absorbance of the TCPP reagent mixture at 432 nm before the addition of a ligand to establish a starting concentration of Cd(II)-TCPP. After this starting concentration is found, 20 µL of a concentrated stock solution of ligand is added, and the absorbance at the 432 nm Cd(II)-TCPP peak is measured again. The concentration of ligand (either citrate or pyrophosphate) in the stock

![Graph showing absorbance vs. [TCPP] (M)](image)

**Figure 2.4** – A calibration plot for the absorbance of TCPP at 432 nm.
solutions were 10 mM, 5 mM, or 100 µM. The dilution effect of adding 20 µL of a ligand solution to 2.98 mL of TCPP reagent solution is minimal; that is, the loss in height of any absorbance peak in the solution due to dilution alone will be less than 1% of the total peak height. As such, this was assumed to be negligible and not factored into our calculations.

The effect of the destruction of the Cd(II)-TCPP on the rate of Mn(III)-TCPP complex formation from Mn(II) and (III) was also studied. To do this, a solution of TCPP reagent was made as described above. For Mn(II), 20 µL of ligand stock solution were added as described above, at the same time as 20 µL of a 1.5mM Mn(II) stock solution. For Mn(III), a stock solution of 1.5 mM Mn(III) stabilized by either 100 mM, 50 mM or 10 mM ligand was made for both pyrophosphate and citrate. After the addition of the manganese and ligand solutions, the rate of growth of the Mn(III)-TCPP absorbance peak at 468 nm was measured over the course of 10 minutes, using the same method and modeling technique detailed in section 2.3.

Figure 2.5 – A broken filter (right) versus an intact filter (left), shown at 40x magnification.

2.7 - Filter Treatment

Filtering to remove colloidal material is crucial in understanding manganese speciation. As colloidal manganese passes through a 0.2 µm filter, filtration with 0.02 µm Anotop filters is required to distinguish colloidal from truly dissolved species. Filters were rinsed with ~5 mL of 0.1 M hydrochloric acid and 15 mL of deionized water before use, or with ~2 mL of experimental solution. Anotop filters cannot be operated under high pressures, or the filter membrane may break as illustrated in Figure 2.5. In order to avoid damaging the filter, a large syringe size (greater than 10 mL syringe volume) must be used to filter the solution.
CHAPTER 3
RESULTS AND DISCUSSION

The purpose of this work was to examine if SRFA could stabilize manganese in the environment, as Mn(III) requires stabilization by a ligand to persist in natural waters, and humic substances such as SRFA are an ubiquitous group of compounds in natural waters with the ability to chelate many different metals. After mixing Mn(II) with permanganate to synthesize Mn(III) in our experimental solutions (see section 2.4), Mn(II) was measured by TCPP at over 60% of the total manganese within the first few minutes, as shown in Figure 3.1. The presence of a slow manganese species was also noted (Figure 3.1), which we needed to positively identify.

3.1 - Mn(III) stability in SRFA solutions

Table 3.1—Measured Mn(II) and slow manganese concentrations for an Mn(III)/SRFA experimental solution filtered with various filter sizes. All data was collected on the same day.

<table>
<thead>
<tr>
<th></th>
<th>0.02 µm filtered</th>
<th>0.22 µm filtered</th>
<th>0.45 µm filtered</th>
<th>Unfiltered</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Mn²⁺] (µM)</td>
<td>78.3</td>
<td>79.8</td>
<td>81.0</td>
<td>80.4</td>
</tr>
<tr>
<td>[Mn_{slow}] (µM)</td>
<td>0</td>
<td>11.1</td>
<td>13.0</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Filtration of the solution with 0.22 and 0.45 µm filters resulted in no visible change in solution color, while 0.02 filtration did remove the color in solution. Filtration also resulted in a clear change in the species present, as shown in Table 3.1. Filtration with a 0.02 µm filter removed most or all of the slow manganese species (shown in Figure 3.2 and in Table 3.4), while 0.22 or 0.45 µm filtration removed only a small portion of the slow manganese species.

Mn(III) solutions prepared with and without SRFA were visually compared. When first prepared, both Mn(III) and Mn(III)/SRFA solutions were a clear, light orange yellow devoid of visible particles, as shown in Figure 3.3a. However, overnight the precipitation of manganese oxide particles was observed in the solution without SRFA, along with a loss of the yellow-orange solution color (Figure 3.3b).
The high concentration of Mn(II) in solution may indicate several fates for Mn(III). There is the possibility that most of the Mn(III) is chelated by SRFA, (perhaps as a colloidal compound) and that a large portion of it is quickly reduced by the SRFA to Mn(II). The Mn(III) may have also disproportionated, by the following reaction:

\[
2Mn^{3+} + 2H_2O \rightarrow Mn^{2+} + MnO_2 + 4H^+
\]

Stoichiometrically, the high concentration of Mn(II) (greater than 50% of the total manganese even at the earliest time points) indicates that disproportionation likely took place.

For either scenario, the slow manganese species observed may be colloidal Mn(III) which may have been stabilized by the SRFA, or colloidal Mn(IV) oxides. Filtration of the Mn(III)/SRFA solution shows that these particles largely fall in the 0.02-0.2µm size range (Table 3.1). The removal of the slow manganese species during filtration cannot be attributed to the reduction of Mn(III) to Mn(II) in the filter, since there was no significant increase in measured Mn(II) concentrations. This slow manganese species also cannot be attributed to a drift in the baseline TCPP reagent concentration, as the filtered solution did not show the same slow
increase in absorbance. It is also clear that the colloidal species was being reduced to Mn(II) over time (Figure 3.1). This reduction can be expected for either colloidal manganese oxides or colloidal Mn(III)-SRFA complexes; however in our opinion the former is more likely, as fulvic acids have been noted to both stabilize and reductively dissolve colloidal metal oxides (Waite et al., 1988; Wilkinson et al., 1997) and Mn(III)-SRFA is more likely to be a dissolved complex than a colloidal one. Thus, we attribute the slow manganese species to colloidal manganese oxides stabilized in the suspension by SRFA. The immediate formation of Mn(II) at a concentration exceeding more than half the total manganese would indicate that little or no Mn(III) was stabilized by SRFA and that the colloidal manganese was mostly MnO₂.

The measured rate constant for our colloidal manganese (Table 3.2) is comparable to the reported rate constant for Mn(III) in Madison et al. (2013), which varied from 0.0037-0.0054 sec⁻¹. The measured Mn(II) rate constants from Madison et al. (2013) ranged from 0.019-0.033 sec⁻¹. These values are lower than our measured Mn(II) reaction rate and their laboratory measurements of Mn(II), which they attribute to interference from high levels of chloride in their field samples.
Table 3.2 - Measured manganese concentrations and kinetic rate constants.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>[Mn$^{2+}$] (µM)</th>
<th>[Mn$_{Slow}$] (µM)</th>
<th>k-Mn$^{2+}$ (1/sec)</th>
<th>k-Mn$_{Slow}$ (1/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.033</td>
<td>60.0</td>
<td>18.4</td>
<td>0.0905</td>
<td>0.0037</td>
</tr>
<tr>
<td>0.517</td>
<td>62.1</td>
<td>16.2</td>
<td>0.0749</td>
<td>0.0037</td>
</tr>
<tr>
<td>1.017</td>
<td>63.3</td>
<td>13.7</td>
<td>0.0795</td>
<td>0.0054</td>
</tr>
<tr>
<td>22.500</td>
<td>69.9</td>
<td>12.2</td>
<td>0.0806</td>
<td>0.0044</td>
</tr>
<tr>
<td>98.4</td>
<td>75.3</td>
<td>9.0</td>
<td>0.0906</td>
<td>0.0044</td>
</tr>
<tr>
<td>190</td>
<td>78.6</td>
<td>9.2</td>
<td>0.0768</td>
<td>0.0039</td>
</tr>
</tbody>
</table>

Figure 3.3 – Solutions of Mn(III) without (left) and with SRFA(right) at 30 minutes after solution formation (A) and 48 hours (B).

If colloidal MnO$_2$ does produce a signal that may be interpreted as Mn(III), then past results by Madison et al. (2011, 2013) may need to be investigated further. Madison et al. (2011) synthesized colloidal MnO$_2$ by the reaction of potassium permanganate and sodium thiosulfate, and found no interference with the TCPP method by these colloids. However, it is possible that colloidal MnO$_2$ formed in the presence of SRFA is more reactive to TCPP. Alternatively, these colloids may not be reacting directly with TCPP, but rather being reductively dissolved by the fulvic acid in solution during the TCPP measurement, forming either Mn(II) or (III) which then complexes with the TCPP. This hypothesis is in line with previous observations about the behavior of manganese oxides in the presence of natural organic matter (Waite, et al. 1988).

The work of Oldham et al. (2015) may also have colloidal manganese giving a false positive for Mn(III). The authors again use the kinetic TCPP method, but consider three possibilities for manganese in a sample that has been filtered in a 0.2 µm filter: 1) Only Mn(II) is present, and can be modeled as one species, 2) that the Mn(III) is stabilized by weak ligands
which allow the manganese to complex with TCPP, and 3) that Mn(III) is stabilized by strong ligands which prevent Mn(III) from complexing with TCPP. In the first two cases the manganese speciation can be modeled as either one or two species, as before. The presence of strongly complexed Mn(III) can be confirmed by measuring the concentrations of both manganese species with the kinetic TCPP method, then treating the sample with a reducing agent (hydrogen sulfide) and measuring manganese concentrations again with the kinetic TCPP method. If the total manganese has increased after treatment with hydrogen sulfide, then the Mn(III) was assumed to be present in a complex with a strong ligand. However, it is possible that colloidal manganese less than 0.2 µm in size has been reduced, as Table 3.1 shows that 0.22 µm filtration removes only a small amount of colloidal material.

### 3.2 – Oxidation State Determination

Determining the oxidation state of manganese captured on a 0.02 µm filter would allow for a clearer understanding of the speciation of the colloidal manganese. If Mn(III) dominates, then a colloidal Mn(III)-SRFA complex is likely present on the filter. If Mn(IV) is most common, then a manganese oxide is likely. To address oxidation state of the filter-bound manganese, the DPD method (section 2.5) was used. Results were inconsistent, even though the

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percent Mn(III)</th>
<th>Percent Mn(IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>74.9</td>
<td>25.1</td>
</tr>
<tr>
<td>Sample 2</td>
<td>44.0</td>
<td>56.0</td>
</tr>
</tbody>
</table>

Table 3.3 - The measured composition of manganese captured on a 0.02 µm filter for two runs of the same sample.

These results suggest the presence of considerable amounts of Mn(III) on the filter, but have very poor agreement. While Mn(III) may be present, under-measurement of the Mn(II) concentrations by AA (which was observed) or the failure of the filter membranes as shown in Figure 2.5 likely resulted in such poor data, and as such these data cannot be used to draw any useful conclusions. Measuring too little dissolved manganese will cause an over-estimation the
amount of colloidal manganese found on the filter, resulting in more Mn(III) in the oxidation state measurement than is present.

3.3 - Filter Treatment

Pre-treating the filters with the Mn(III)/SRFA solution resulted in a loss of Mn(II), and the complete removal of the kinetically slow manganese species (Table 3.4). The recovery of Mn(II) in the filtrate increased with time, and there was only a slight difference between the two types of filter treatment, as shown in Figure 3.4.

Table 3.4 – Measured manganese concentrations over time, for filtered and unfiltered samples. No measurable kinetically slow manganese was detected. These data are from the same time series as Figure 3.1.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Filter Treatment</th>
<th>Unfiltered</th>
<th>Filtered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Mn$^{2+}$] (µM)</td>
<td>[Mn$_{Slow}$] (µM)</td>
<td>[Mn$^{2+}$] (µM)</td>
</tr>
<tr>
<td>0.1</td>
<td>Experimental solution</td>
<td>60.0</td>
<td>18.4</td>
</tr>
<tr>
<td>0.5</td>
<td>Experimental solution</td>
<td>62.1</td>
<td>16.2</td>
</tr>
<tr>
<td>1.0</td>
<td>Experimental solution</td>
<td>63.3</td>
<td>13.7</td>
</tr>
<tr>
<td>22.5</td>
<td>Experimental solution</td>
<td>69.9</td>
<td>12.2</td>
</tr>
<tr>
<td>98.4</td>
<td>HCl/DI Water</td>
<td>75.3</td>
<td>9.0</td>
</tr>
<tr>
<td>190.0</td>
<td>HCl/DI Water</td>
<td>78.6</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Simply pre-rinsing the filters with manganese/SRFA solution would theoretically remove any redox species from the filter and eliminate any dilution effects, but recoveries of Mn(II) were consistently low. This may be attributed to ‘fouling’ of the filter by manganese oxides in the case of both SRFA and oxidant pre-treatments, which may prompt sorption of Mn(II) to their surface during filtration, or introduce an osmotic pressure that prevents some of the positively-charged Mn(II) from moving past the charged filter surface (Fitzsimmons & Boyle (2014)). The ability of the colloidal manganese to sorb the Mn(II) appears to decrease as the measured concentration of the kinetically slow manganese species decreases, perhaps because of a decrease in reactivity of the colloidal manganese over time. These issues, coupled with the frequent failure of the filter membrane, meant that experimentation with filters was conducted infrequently, and with great care to dry the filter after treatment, and drive samples through the filter at low pressure to avoid damaging the filter.
Figure 3.4 - The percent recovery of Mn(II) in the filtrate over time. Points in blue are the Mn(III)/SRFA filter treatment, and points in red are the hydrochloric acid/DI water treatment.

3.4 - Effects of Cadmium to Ligand Ratios on the TCPP Kinetic Method

Our research was also going to investigate the ability of citrate to stabilize Mn(III), a simple, redox-active model ligand for SRFA. However, high concentrations of citrate are required to stabilize Mn(III), so the effect that high ligand concentrations have on the TCPP method needed to be examined. Citrate and pyrophosphate concentrations were varied at two different cadmium concentrations, 12 µM and 24 µM. Varying the ratio of cadmium to ligand allowed us to examine how cadmium speciates in a system with TCPP and another strong ligand. Figure 3.5 shows the effect on the Cd(II)-TCPP concentration with increasing ligand concentrations for both pyrophosphate (red) and citrate (blue). The results show that at a cadmium concentration of 6 µM, the concentration of Cd(II)-TCPP can be decreased significantly at low ligand concentrations, while the stoichiometrically correct concentration (12 µM) yields a Cd(II)-TCPP concentration that is stable to much higher ligand concentrations.
Figure 3.5 – Measurements of the Cd(II)-TCPP concentration in solutions with half the normal cadmium concentration (horizontal diamonds) and the full cadmium concentrations (vertical diamonds) at different ligand concentrations (pyrophosphate is red, citrate is blue).

Figure 3.6 – The measured reaction rate constant (k) for solutions of Mn(II) and Mn(III) in the presence of varying concentration of the ligand pyrophosphate. Note that some pyrophosphate is required to stabilize Mn(III), so there is no measurement for 0 µM pyrophosphate in the Mn(III) measurements.
The loss of Cd(II)-TCPP also directly affects the rate of reaction between TCPP and both manganese species. The change in rate is illustrated in Figure 3.6, which shows the measured reaction rate constants (see section 2.3) for both Mn(II) and slow manganese (in this case, added Mn(III)) in a TCPP reagent solution with 6 µM cadmium. Reaction rates of both species fall off with increasing pyrophosphate concentration, and at appreciably high pyrophosphate concentrations the reaction rates for both manganese species become indistinguishable. This means that environments with high ligand concentrations (which would be the most likely to stabilize Mn(III)) may also interfere with the TCPP method’s ability to distinguish Mn(II) from Mn(III), and that special care must be taken to account for the loss of cadmium from the Cd(II)-TCPP complex. Also, the high concentrations of citrate required to stabilize measurable amounts of Mn(III) (200 times the concentration of Mn(III) (Vyas, Van Hoomissen and Voelker, unpublished data)) had a large an effect on the Cd(II)-TCPP concentration in our experiments, which eliminated the possibility of using the TCPP method to explore the citrate-Mn(III) system. While unlikely in nature, the effect of >100µM ligand concentrations in solution are a limiting factor in laboratory work, especially when using ligands which may require high concentrations to effectively stabilize Mn(III).
CHAPTER 4
CONCLUSIONS

Our data do not provide any evidence that Mn(III) in the Mn(III)/SRFA mixture is stabilized by complexation with SRFA on any measurable timescale. Instead, it is more likely that rapid disproportionation of the Mn(III) into Mn(II) and colloidal MnO₂ was observed. These colloidal particles may be mistaken for Mn(III) by the TCPP method, as their kinetic rate constants are similar to kinetic rate constants attributed to Mn(III) in natural waters by Madison et al. (2013).

Much work remains to definitively prove that SRFA is incapable of stabilizing Mn(III). Our approach assumed that some Mn(III) would be stabilized by SRFA if a large quantity of Mn(III) were introduced to a SRFA solution. However, it may be possible that our reaction conditions favored disproportionation and that SRFA can complex with Mn(III) if the Mn(III) is stabilized by another intermediate ligand, such as those discussed in Oldham et al. (2013).

More work on the oxidation state of manganese collected on 0.02 µm filters must be conducted. Determining the bulk oxidation state of these colloidal species would allow us to identify the species as either a colloidal Mn(III)-SRFA complex or colloidal manganese oxides. Transmission electron microscopy may also aid in the identification of these species. A Mn(III)-SRFA colloid would likely image as an amorphous mass, while colloidal manganese oxides may appear crystalline in form.

Other experimentation should focus on the nature and origin of the slow manganese species signal produced by the colloidal Mn(III,IV) oxides. Experiments into whether the manganese oxides are being reduced to Mn(II) or (III) by the SRFA before complexing with TCPP or if the TCPP is directly reducing the oxide should be conducted, as both possibilities are relevant to future studies. A false positive for Mn(III) caused by the direct oxidation of manganese oxides by TCPP would call into question past studies on the presence of dissolved Mn(III), while a signal caused by the reductive dissolution of manganese oxides by humic substances would necessitate the careful removal of these oxides before measuring dissolved Mn(II) or (III). In either case, filtration of a water sample with an appropriately small pore size (0.02 µm) is necessary to avoid this false signal, and accurately detect dissolved Mn(III). To our knowledge, all previous studies of Mn(III) in the environment using the TCPP method have been
conducted with samples filtered with either 0.45 or 0.20 µm filters. In the case of SRFA acting as the reductant, the TCPP method may be employed to study the rates of manganese oxide reduction by organic compounds, provided the final product of the manganese oxide reduction is Mn(II).
REFERENCES CITED


Kilian, K., & Pyrzyńska, K. (2003). Spectrophotometric study of Cd(II), Pb(II), Hg(II) and Zn(II) complexes with 5,10,15,20-tetrakis(4-carboxylphenyl)porphyrin. Talanta, 60(4), 669–78. DOI:10.1016/S0039-9140(03)00149-8


APPENDIX A
Mathematica Code

The Mathematica code used to solve for Mn(II) and $Mn_{slow}$ concentrations and kinetic rate constants, as well as to produce a graph of the modeled results.

Clear[file]
Clear[data]
Print["-------------------------------------- File loaded: "]
file = "Insert file path here"
data = Import[file];
time = data[[All, 1]];  
abs = data[[All, 2]];
baseline = data[[1, 2]];

p1 = ListPlot[data, PlotStyle -> Green,
    PlotRange -> {{0, Max[data]*1.1}, {0, (Max[data[[All, 2]]]*1.1)}},
    PlotLegends -> {"Data"}];
Clear[MnSlow]
Clear[k3]
Clear[Mn2]
Clear[k2]

model = ((MnSlow*(1 - Exp[-(kslow)*x]))+(Mn2*(1-Exp[-(k2)*x]))) + baseline;
Print["Model Form: ", model];
fit = FindFit[data, {model}, {k2,Mn2,kslow,MnSlow}, x, MaxIterations -> 1000]
Print["-------------- RESULT ------- ------- "]
p2 = Plot[Evaluate[model /. fit], {x, 0, Max[data]*1.1}, PlotRange -> Full, PlotStyle -> Gray,
    PlotLegends -> {"Model"}];
values = {k2,Mn2,kslow,MnSlow} /. fit;
p3 = Plot[{values[2]*(1 - Exp[-(values[1])*x])}, {x, 0, Max[data]*1.1}, PlotRange -> Full,
    PlotStyle -> Red, PlotLegends -> {"Mn2+"}];
p4 = Plot[{values[4]*(1 - Exp[-(values[3])*x])}, {x, 0, Max[data]*1.1}, PlotRange -> Full,
    PlotStyle -> Blue, PlotLegends -> {"Mn-slow"}];
Show[p1, p2, p3, p4, AxesLabel -> {Time, Absorbance}]
Print["Mn2+ Conc: ", (values[[2]])/95400] // N, " M"]
Print["MnSlow Conc: ", (values[[4]])/95400] // N, " M"]
Print["Mn2+ k Value: ", (values[[1]])] // N, " (1/sec)"]
Print["MnSlow k Value: ", (values[[3]])] // N, " (1/sec)"]