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Influence of Salinity on Copper-Ligand Formation

Constants

by:

Barnawi, Fatimah

(Master's degree, Wilfrid Laurier University, 2019)

THESIS

Submitted to the Department of Chemistry and Biochemistry

in partial fulfilment of requirement for

Master of science in Chemistry

Wilfrid Laurier University

Waterloo, Ontario, Canada

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Abstract

Natural Organic Matter (NOM) is known to reduce metal, such as copper, toxicity in aquatic environments. Copper is essential for organisms, but elevated concentrations of dissolved copper can potentially be toxic. The toxicity of copper is related to its bioavailability, which is influenced by toxicity modifying factors, such as NOM (quantified as Dissolved Organic Carbon (DOC) in mg C/L), alkalinity, pH and major cation and anion concentrations. The principles are the same for fresh and saltwater, but the influence of high salt concentrations, such as in estuaries, and associated activity corrections, can modify NOM complexation of metals (i.e., modify logK values). The magnitude of such corrections has not been systematically studied in the context of risk assessment tools such as the Biotic Ligand Model (BLM), and the mathematical tools currently used to correct logK values for salt effects have not been fully tested experimentally. The purpose of this study is to address these gaps by determining logK values across a range of salinities for copper association with salicylic acid as a proxy to NOM. This study used varying concentrations of salt, either as artificial seawater (10 to 100%) or simply as sodium sulfate salt (Na_2SO_4). In these salt solutions, logK values for copper binding to salicylic acid were determined using fluorescence quenching titrations and nonlinear regression (the so-called Ryan-Weber method). Salicylic acid is a well-defined compound, so these logK values were compared to certified values from the National Institute of Standards and Technology (NIST) measured at different ionic strengths. In addition, comparisons of logK values were made between measured values, NIST interpolations, and calculations using the extended Debye-Huckel (DH) equation. The results show that the extended DH calculation has a great agreement with the certified NIST values. However, the fluorescence quenching method has disagreement compared to other methods. It was off by an order magnitude. As a future study, it is better to test different methods such as Ion Selective Electrode and how the results match others.

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List of Abbreviations and Symbols

ASW	Artificial Seawater
BL	Biotic Ligand
BLM	Biotic Ligand Model
Cu	Copper
Cu_T	Total Copper
Cu-Sal	Copper Binding to Salicylic Acid
DH	Deybay-Huckle
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EC50	Half maximal effective concentration
FQ	Fluorescence Quenching
NOM	Natural Organic Matter
HSal	Salicylic acid
ISE	Ion Selective Electrode
LogK	Equilibrium Constant
L_T	Binding Capacity (Total Ligand)
M	Metal
NIST	National Institute of Standards and Technology
OECD	Organization for Environment Quality Guidelines
OM	Organic Matter
pH	Negative Log of the Hydrogen Ion Concentration (-log [H ⁺])

RW	Ryan-Weber
SPAWAR	The Space and Naval Warfare Systems Command
UV	Ultraviolet Light
μ	Ionic strength
γ	Activity Coefficient

Chapter 1

Introduction

1.1 Overview

Metals play a significant role in aquatic environmental pollution. Many metals are essential for aquatic organisms, but elevated concentrations of dissolved metal cations, including Cu^{2+} and Ni^{2+} , could seriously affect the aquatic environment due to their potential toxicity at higher concentrations (Chapman et al., 1998; Howard, 1998; Mason, 2013; Nadella et al., 2009; Smith et al., 2017; Smith et al., 2011).

1.2 Copper

Copper (Cu) is found in water in different forms including complexes with Dissolved Organic Matter (DOM) as well as inorganic species (*e.g.*, $\text{Cu}(\text{OH})^+$, CuCl^+ , and CuHCO_3) (Kogut & Voelker, 2001; Sunda & Hanson, 1979). Both the organic and inorganic complexed forms have limited availability $\text{Cu}(\text{OH})^+$ to be toxic to organisms (Kogut & Voelker, 2001; Sunda & Hanson, 1979). Consequently, free copper (Cu^{2+}) is widely used as a predictor of toxicity, since it can be proportional to the bioavailable fraction of total copper (Glasby & Schulz, 1999; González-Dávila et al., 2009). Also, free copper can bind with the biotic ligand (BL) on organisms (*e.g.*, gill) and potentially block

ion channels and potentially causing death by ion regulatory disturbance (Di Toro et al., 2001; Grosell & Wood, 2002).

The Biotic Ligand Model (BLM) can be used to predict copper toxicity based on calculations of copper speciation. From BLM perspective, anything that prevents copper accumulation at the BL is expected to decrease Cu toxicity. For instance, alkalinity has an influence on metal toxicity. In Fig.1, when the alkalinity increases cations like Na^+ and Ca^{2+} increase, the toxicity decreases because these cations will reduce the binding between metal (e.g. Cu^{2+}) and BLs of the organism. Also, increasing alkalinity increase anions species, such as (bicarbonate HCO_3^- and carbonate CO_3^{2-}), which will reduce the toxicity of metals because metals like Ca^{2+} will bind with these anions in solution instead of binding to the BL.

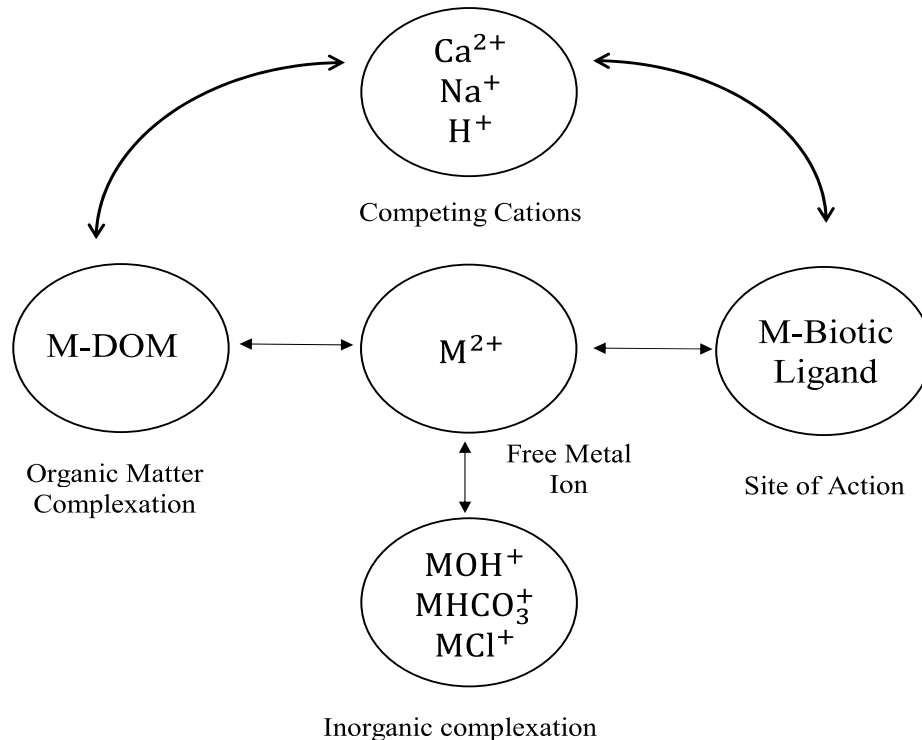


Figure 1. Schematic diagram of the Biotic Ligand Model (Adapted from Di Toro *et al.*, 2001).

1.3 Predicting the toxicity and bioavailability of metals

Many studies predict the toxicity and bioavailability of dissolved copper in water by using the BLM (Figure 1) (Di Toro *et al.*, 2001; Mart *et al.*, 1985; Pagenkopf, 1983; Paquin *et al.*, 2002; Smith *et al.*, 2017; Sunda & Hanson, 1979; Wang *et al.*, 2012). The BLM could be defined as a chemical equilibrium-based model that depends on the log K values for reactions involving metals, ligands and competing species (Figure 1) (Di Toro *et al.*, 2001). The BLM is a predictive tool to estimate toxicity as a function of water quality variables and can be used to assess the risk of aqueous metals (Engel & Sunda, 1979; D. S. Smith *et al.*, 2017; Wang *et al.*, 2012). The bioavailability of metals and ultimately toxicity is predicted by toxicity modifying factors, such as pH, dissolved organic matter, sodium, cation concentration, alkalinity, and other factors (Paquin *et al.*, 2002). Predictions of the binding between BL and dissolved metals (*e.g.*, Cu²⁺) aids in the establishment of the environmental regulation for surface water. Furthermore, the BLM allows regulators to take site specific chemical characteristic of the receiving water into account when assessing risk of copper discharge.

1.4 Estuary

1.4.1 The estuarine environment

There are several types of surface waters potentially impacted by metal pollution. Depending on salinity, water might be classified as salt water or fresh water, or intermediate between the two, such intermediate salinity water is referred to as estuarine. The pH range of salt water is between 7.8 and 8.3 (Millero, 2001); however, fresh water contains a wider range of pH

between 5.5 to 7.5 (Shirlie, 2018). An estuarine system has pH range between 7.5 and 8.8 (Day et al., 1989). The estuary system is classified as a special type of water because it contains a high range of various salinity and pH levels where the fresh river water and marine salt water mix (Whitfield, 1999). The multiple levels of salinity have influences on several toxicity modifying factors of the BLM such as pH, alkalinity, ionic strength and dissolved organic matter (DOM). Salinity in particular will modify the K values that form the basis of the BLM, and the model needs to be modified to take this into account. Thus, it is important to include the role of salinity in BLMs. Currently, there is limited resources on how best to make these corrections. This thesis will address this important data gap.

1.4.2 Copper toxicity to selected marine invertebrates

There is a study by Smith et al. (2010) that reports a comparison of some organisms present in saltwater and their sensitivities to copper. These invertebrate species include *Mytilus galloprovincialis*, *Strongylocentrotus purpuratus*, *Eurytemora affinis*, *Crassostrea virginica*, *Mytilus edulis*, *Eurytemora affinis*, and *Dendraster excentricus*. The most sensitive species was *Mytilus galloprovincialis*, which was affected by a low amount of copper that was $EC_{50} \text{ (mg Cu/L)} = 8.53 \text{ DOC (mg C/L)}^{0.87}$. The equation of the prediction for EC_{50} was used as a function of DOC. The EC_{50} is the half-maximal effective concentration of the agonist that raises a response halfway between the lowest line and the highest response of the curve. Compared to organisms, they are classified based on sensitivity factors as more or less sensitive depending on the conditions. The study focused on measuring the quality of various Natural Organic Matter (presented as Dissolved Organic Carbon (DOC)) as inhibitor to the toxicity of free copper in salt water, including different concentrations of DOC. Although the researcher found that there is a

relationship between Cu^{2+} toxicity and DOC, there are no significant differences between sources of DOC.

1.4.3 The influence of salinity on BLM

The BLM has different behavior based on salinity. Each type of water has different quality standards. For instance, the estuaries system is a special type of water because it has various salinity levels. These various salinity levels have influences on several factors present in the system such as pH, alkalinity, ionic strength, and metal speciations. These factors affect the results of BLM. Moreover, one of these factors that affects the BLM is ionic strength. Ions can make the ionic atmosphere, which can prevent the potential reactivity of the species, and that will affect the results of BLM. For example, using a saturated solution of CaSO_4 in distilled water with K_{sp} of 2.4×10^{-5} , it showed a solubility of 0.015 M. However, adding inert salt, such as KNO_3 will increase the solubility of the sparingly soluble salt.

1.5 Thesis objective

The objective of this research is to experimentally characterize the effect of various ionic strengths on copper binding to salicylic acid ($\log K$ values for formation of copper complexes) in simulated estuary waters and determine, using modeling, the impacts of this variable ionic strength estuary system on BLM predictions. This prediction of variable ionic strength is testing two activity correction approaches, which are fluorescence quenching and extended Debye-Huckel (DH) that is compared to measured values from National Institute of Standard and Technology (NIST).

1.6 The significance of the study

BLM depends on logK values, and logK values are depending on salinity. The current BLM framework can easily be modified to use the extended DH to correct for ionic strength effects. Such modifications need to be validated experimentally though. This study assesses the effect of various salt concentrations on the measured and modelled logK values and can help inform future saltwater developments of the BLM. There are two main aspects of this study:

- 1) Is the DH correction appropriate for estuarine and saltwater BLMs?
- 2) Is fluorescence quenching an appropriate method to measure the salinity dependence of the formation constant logK values of copper binding to salicylic acid?

This study is used the mathematical K_f correction using extended DH equation for salicylic acid binding copper. It is important to point out that DH can be used only for a small molecule, such as salicylic acid, but it is not possible to use DH in large molecules, such as NOM. Thus, fluorescence quenching method is going to be used to test its effectiveness to measure the variable ionic strength dependent K_f values for NOM-Cu complex formation.

1.7 The theory

1.7.1 Activity correction

The activity of the species is the thermodynamically reactive concentrations of these species. Equilibrium constant are only truly constant when expressed in activity unit. The activity for species C is present in equation (1) as:

$$\mathcal{A}_C = \gamma_C [C] \quad (1)$$

where \mathcal{A}_C the activity for species C. γ_C is the activity coefficient of species C, and $[C]$ is the concentration of the species C in molar units. The activity correction concept is used in the equilibrium constant equation (*i.e.* law of mass action). To correct the concentration-based equilibrium constant equation including activity, there are several steps that must be applied. An equilibrium general reaction is presented in equation (2):



The equilibrium constant for reaction (2) without including any activity correction is in equation (3):

$$K_{\text{conc}} = \frac{[C]^c [D]^d}{[A]^a [B]^b} \quad (3)$$

Equation (3) is defined the equilibrium constant (K_{conc}) values in terms of concentration, in molar units. The result of (K) value in equation (3) is only constant at a constant ionic strength. The ionic strength (μ) is related to the concentration of the species in the solution and charge, and it is used to find the total concentration of ions in the solution. Ionic strength, (μ) is defined in equation (4):

$$\mu = \frac{1}{2} \sum c_i z_i^2 \quad (4)$$

where z_i is the charge of the species, and c_i is the concentration of the *i*th species.

If the concentrations are replaced by activities in the equilibrium constant, it represents K_{thermo} (equation 5). Modifying equation (3) by including the activity coefficient γ , which is a function of ionic strength, gives a true constant independent of ionic strength that is present in equation (5).

$$K_{\text{thermo}} = \frac{\mathcal{A}_C^c \mathcal{A}_D^d}{\mathcal{A}_A^a \mathcal{A}_B^b} = \frac{[C]^c \gamma_C^c [D]^d \gamma_D^d}{[A]^a \gamma_A^a [B]^b \gamma_B^b} \quad (5)$$

Equation (5) is presents the thermodynamic equilibrium constant including activity correction. By rearranging equation (5), K_{thermo} will be in equation (6) that includes a multiplication of equation (3) with are the activity coefficients:

$$K_{\text{thermo}} = \frac{[C]^c [D]^d}{[A]^a [B]^b} \cdot \frac{\gamma_C^c \gamma_D^d}{\gamma_A^a \gamma_B^b} \quad (6)$$

To simplify, the following expression can be derived by substituting K_{conc} (equation 3) into the equation (7):

$$K_{\text{thermo}} = K_{\text{conc}} \frac{\gamma_C^c \gamma_D^d}{\gamma_A^a \gamma_B^b} \quad (7)$$

Equation (7) presents the expression for K values including activities corrections. The γ values are constant for a constant ionic strength; thus, K_{conc} is constant at a constant ionic strength (*i.e.*, salinity). To estimate the activity coefficient (γ), in equation (7), one of the activity correction models such as extended Debye-Huckel equation, Davies equation, and Pitzer equation could be used (Harris, 2010). Both the Davies and Pitzer equation are explained in 3.2.5 LogK values for speciation in seawater section.

This study will focus on using the extended Debye-Huckel equation (equation 8) to correct the logK values used in the BLM. This extended Debye-Huckel equation is appropriate and easy to implement as suggested by the Space and Naval Warfare Systems Command (SPAWAR) in a recent study (Chadwick et al., 2008) to solve K_{conc} values, as used by BLM, in equation 8.

$$\log \gamma = \frac{-0.51 z^2 \sqrt{\mu}}{1 + (\alpha \sqrt{\mu}/305)} \quad (8)$$

where z is a charge for the species, and μ is the ionic strength mol/L of the solutions. α is the ion size, in picometers (pm). Values for α are determined experimentally and tabulated (Harris, 2010).

1.8 Analytical methods

1.8.1 Fluorescence

Fluorescence occurs as the emission of a photon during a transition between states with the same spin quantum numbers (*e.g.* $S_1 \rightarrow S_0$). The fluorescence phenomenon is measured as emitted light from sample that absorbed light. This emitted light has a wavelength longer (lower energy) than the absorbed wavelength (high energy). Fluorescence is instantaneous, when the excitation light source is turned off, the emitted light fluorescence will stop immediately (Harris, 2010).

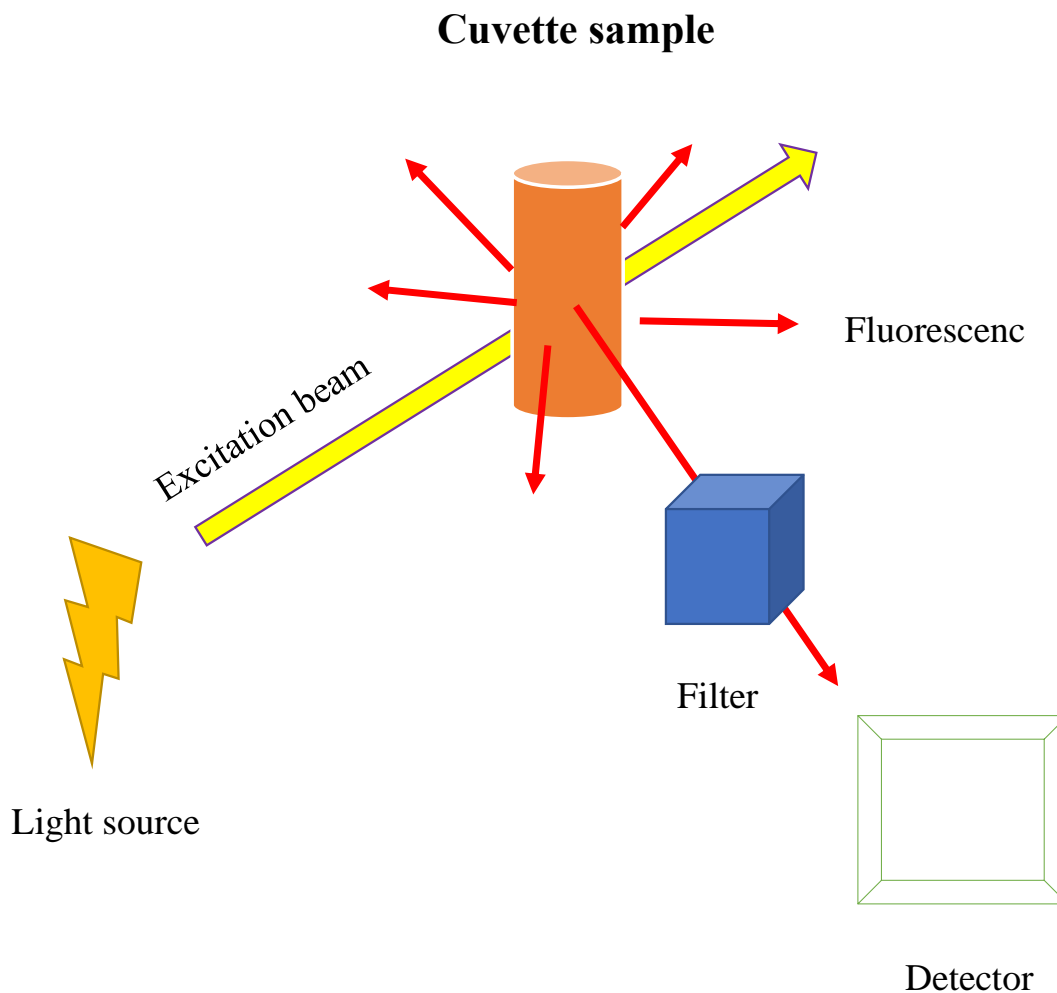


Figure 2. The components of a fluorescence spectrometer (adapted from Hooijschuur, 2018).

The components of the fluorescence spectroscopy are light source, excitation and emission monochromator, sample holder (cuvette), and detector (Fig. 2). The light source typically emits light in the ultraviolet range. This light has multiple wavelengths, and the excitation monochromator selects one specific wavelength (λ_{ex}). This specific wavelength (λ_{ex}) enters into the liquid sample in a cuvette, and part of the light is absorbed by the sample. Additionally, when the sample absorbs the UV light, which means absorbing a photon, the molecules in the sample become excited, and there are several events that may occur. One of

these possible events is the fluorescent phenomenon. Figure 3 illustrates the transformation of electrons between the ground state and excited state from Jablonski diagram (Harris, 2010, Fig. 3).

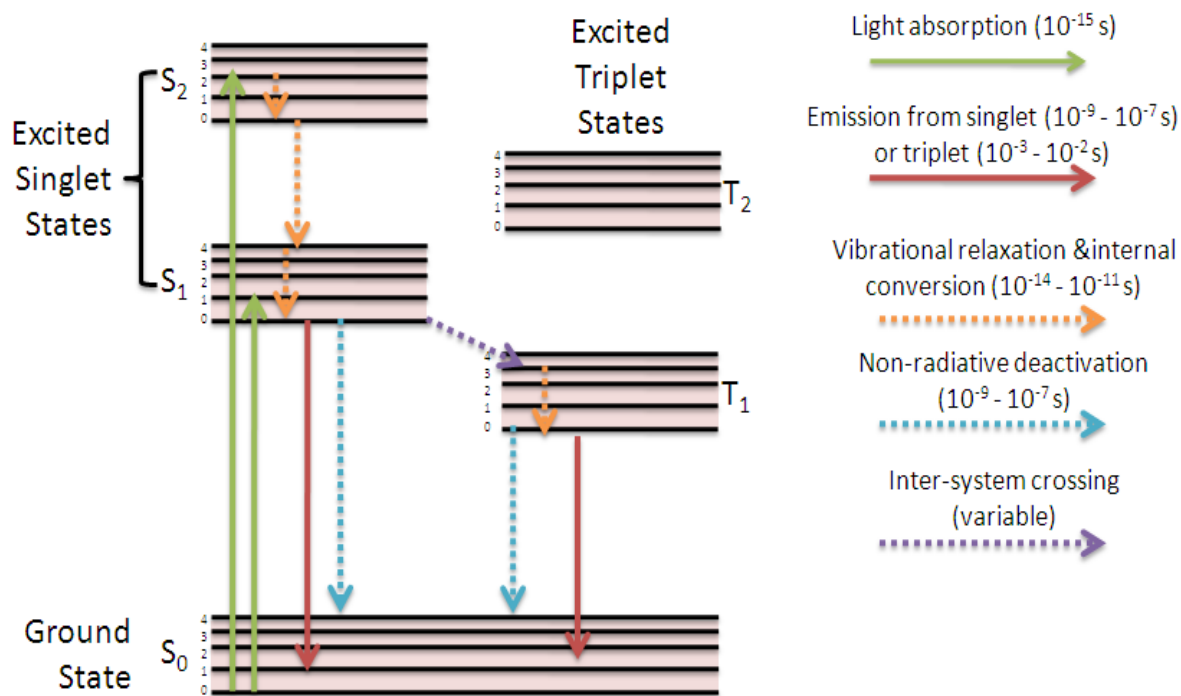


Figure 3. Jablonski diagram for radiative and non-radiative transitions (Adapted from Harris, 2010).

Similar to excitation, the emitted light has various wavelengths, and the emission monochromator selects one for intensity measurement at the detector (λ_{em}). The detector is measured the emitted radiation, and the results of the fluorescence are shown as a wavelength that is described the relationship between the intensity of the fluorescence versus the wavelength in nanometer (nm) unit (Figure 2).

1.8.2 Fluorescence Quenching

Fluorescence quenching technique (FQ) is a useful tool that determines both the density of sites (L_T) and the conditional stability (K') of multi-sites for metal-natural organic matter (NOM) interactions (Smith & Kramer, 2000). The fluorescence of NOM can be quenched in the presence of metal cations such as Cu^{2+} (da Silva, Machado, Oliveira, & Pinto, 1998).

Fluorescence can monitor the interaction between metal and ligand (ML) and the ligand (L) species. During the titration, each addition of metal will decrease the intensity of the fluorescence at a given excitation/emission wavelength. The decrease of the intensity of the fluorescence at a given excitation/emission wavelength will be dependent on the amount of bound ligand. For example, humic substances can complex Cu in natural waters (Merdy et al., 2012). The range of fluorescence emission for humic substances is between 400 and 500 nm (Coble, 1996).

A plot of the fluorescence versus added metal can be fit by using the Ryan-Weber (RW) equation (Ryan & Weber, 1982) (equation 9) below. There is a study by Smith and Kramer (2000) assumes the responsibility of the fluorescence as a linear line with the concentrations, so a linear fit line was made for each experiment separately. Also, the tableau (Appendix 1) of the seawater species was considered in the measurements as well to get logK values metal-ligand complex formation.

$$I = \left(\frac{I_{ML} - 100}{2KL_T} \right) \left[(KL_T + KM_T + 1) - \sqrt{(KL_T + KM_T + 1)^2 - 4K^2L_TM_T} \right] + 100 \quad (9)$$

I = the percentage of fluorescence intensity $\frac{I}{I_0} \times 100$.

I_{ML} = the intensity of the fluorescence when all ligand bounds to metal.

K = the conditional stability constant.

L_T = the total ligand concentration.

M_T = the total metal concentration.

The relevant fitted parameter from the RW equation are K and L_T values. Thus, for different salinities, it is possible to measure K_{conc} using this method.

Moreover, fluorescence can quench statically or dynamically. There is a collision in the excited state for electrons, and they can be returned back to the ground state in two ways as a radiative or non-radiative. The radiative way of the electrons is by losing energy through emitting light; however, the non-radiative way is going through the pathway and most of the electrons do that, which means that they do not fluoresce. One of the non-radiative ways is collisions in the excited state that collides with copper. Copper is a ground state complex, and it is reducing fluorescence as a static quenching. However, there is some contribution that can be quenched with the fluorescence as a dynamic quenching, which affects the results of $\log K$ values.

Chapter 2

Materials and Methods

This study determined the influence of varying ionic strength on the binding of copper to simulated organic matter (OM) in an estuarine system. Salicylic acid was used for this simulation as a ligand because it is a proxy of NOM since it has the same functional groups (carboxylic and phenolic). Fluorescence quenching (Aqua-log machine) was used to determine log K values as a function of salinity. All experiments were used ultra-pure water (MilliQ water) that has a resistivity of 18.2 Ω .

2.1 Synthetic seawater

Artificial seawater (ASW) was prepared according to the method provided by the Organization for Economic Co-operation and Development in table 1 (Annex 10, 2007; OECD, 2001). The salinity range of ASW is between 0.5 to 35 g/L.

Table 1. The species present in seawater with their concentrations in molar unit from (OECD) to create an artificial seawater in 1L of MilliQ.

Species		Concentrations
Sodium fluoride	(NaF) ^a	3 mg.L ⁻¹
Strontium chloride hexahydrate	(SrCl ₂ . 6H ₂ O) ^a	20 mg.L ⁻¹

Boric acid	$(\text{H}_3\text{BO}_3)^{\text{b}}$	$30 \text{ mg}\cdot\text{L}^{-1}$
Potassium bromide	$(\text{KBr})^{\text{c}}$	$100 \text{ mg}\cdot\text{L}^{-1}$
Potassium chloride	$(\text{KCl})^{\text{b}}$	$700 \text{ mg}\cdot\text{L}^{-1}$
Calcium chloride dihydrate	$(\text{CaCl}_2\cdot 2\text{H}_2\text{O})^{\text{a}}$	$1.47 \text{ g}\cdot\text{L}^{-1}$
Sodium sulfate	$(\text{Na}_2\cdot\text{SO}_4)^{\text{a}}$	$4.0 \text{ g}\cdot\text{L}^{-1}$
Magnesium chloride hexahydrate	$(\text{MgCl}_2\cdot 6\text{H}_2\text{O})^{\text{c}}$	$10.78 \text{ g}\cdot\text{L}^{-1}$
Sodium chloride	$(\text{NaCl})^{\text{a}}$	$23.5 \text{ g}\cdot\text{L}^{-1}$
Sodium metasilicate nonahydrate	$(\text{Na}_2\text{SiO}_3\cdot 9\text{H}_2\text{O})^{\text{a}}$	$20 \text{ mg}\cdot\text{L}^{-1}$
Sodium bicarbonate	$(\text{NaHCO}_3)^{\text{d}}$	$200 \text{ mg}\cdot\text{L}^{-1}$

^a Fisher Scientific (New Jersey, NY), ^b Sigma Aldrich (St. Louis, MO), ^c BDH (West Chester PA), ^d EMD Chemicals (Gibstown, NJ)

2.2 Copper

Copper stock solution was made from an ACS grade of cupric sulfate pentahydrate $\text{CuSO}_4\cdot 5\text{H}_2\text{O}_{(\text{s})}$. The initial stock was made in $0.1 \text{ mol}\cdot\text{L}^{-1}$ of copper. Then, the standard solutions were made from the dilution of the first stock. These standard solutions were 100, 1000, and 10000 μM of copper, and they were stored in acid-washed, sealed plastic bottles (HDPE).

2.3 Salicylic acid

Salicylic acid $\text{C}_7\text{H}_6\text{O}_3_{(\text{s})}$ (ACS grade, 99.9% pure, Fisher Chemical) was used as a ligand to bind copper. The initial stock standard was made in $0.1 \text{ mol}\cdot\text{L}^{-1}$. It was stored in acid-washed,

sealed plastic bottles (HDPE). Then, 10 μM of salicylic acid was used in each experiment. The structure of salicylic acid is in figure 4.

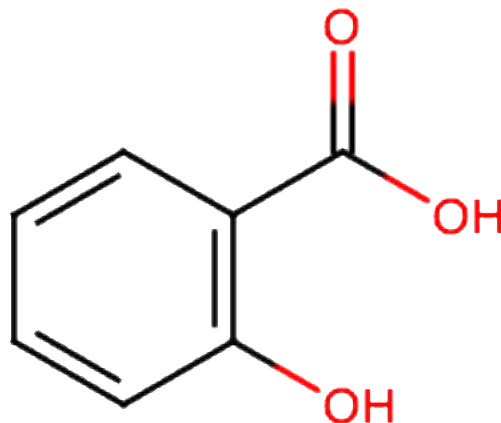


Figure 4. Molecular structures of Salicylic acid.

2.4 Titration

The ASW solution was used to create 100%, 75%, 50%, and 25% seawater samples by dilution with ultrapure water. These samples contained 10 $\mu\text{mol. L}^{-1}$ of salicylic acid. The pH was adjusted to 8.0 by using the dilution of either hydrochloric acid $\text{HCl}_{(\text{aq})}$ or sodium hydroxide $\text{NaOH}_{(\text{aq})}$ as needed. The salt concentrations included in this study in 0.01, 0.05, 0.25, 0.5, and 1 mol. L^{-1} of sodium sulfate anhydrous salt $\text{Na}_2\text{SO}_{4(\text{s})}$ (ACS grade, 99.9% pure, Fisher Chemical). In addition, the solution was titrated by copper standards that are discussed above, and the range of the total concentrations of copper was added from 0 to 350 $\mu\text{mol. L}^{-1}$. Each addition of copper was equilibrated overnight in the samples that contained 10 $\mu\text{mol. L}^{-1}$ of

salicylic acid and the specified concentration of ASW before the fluorescence was measured, to allow sufficient time for copper and ligand to bind to each other (Smith & Kramer, 1998). twenty-four hours equilibration is standard in BLM experiments. Then, part of the solution for each sample was taken into a quartz cuvette to measure the sample using the fluorescence spectrometer (model, manufacturer). Additionally, all experiments were performed at room temperature, which was measured the bioavailability of copper in each sample at 25 ± 0.2 °C.

2.5 Methods

There were three methods that were used in this study to measure the formation constant of copper binding to salicylic acid in variable salt concentrations. These methods were (1) experimental determination of the fluorescence quenching, (2) NIST interpolation and (3) theoretical correction of zero ionic strength.

2.5.1 Experimental Determination

For fluorescent salicylic acid, K_f values were determined experimentally using the fluorescence quenching technique. Each trial was prepared at various salt concentrations. The fluorescence spectra were measured for each sample after each addition of copper. Then, the fluorescence intensity at the peak was used to determine I/I_0 (equation 9) as a function of the total copper added. The steepness of the curves was determined and using nonlinear regression of the RW equation the $\log K$ values were predicted for each of these determinations.

2.5.2 NIST Interpolation

National Institute of Standards and Technology has measured tabulated standard values of K_{conc} (equation 3) for copper binding to salicylic acid. These measured values were in variable ionic strength in a range of 0.0 to 1 mol/L (NIST, 2010). The logK values from NIST were interpolated between these values because there is an assumption that the interpolated line is determined logK for any ionic strength from 0.0 to 1.0 even though these values were not measured directly. The correction of NIST measured values was used as a code in Matlab program (Appendice 3) that not only includes the correction of Cu-Sal but also includes the other reactions for the species present in seawater. The reason for including the correction for other reactions is to determine the corrected logK for the reaction between Cu-Sal.

2.5.3 Theoretical Correction of Zero Ionic Strength

The theoretical correction of zero ionic strength was used the formation constant of the binding between copper and salicylic acid. This correction used the activity coefficient that is determined from the DH equation, which is related to the ionic strength and ion size. Then, all of these activities were multiplied with the formation constant, and that predicted the corrected logK values (Appendice 2), which explains the matlab example code of the DH calculations.

2.6 Matlab

Matlab was used to complete the determination for all modelling sections. Matlab is a software that has a high-performance for a computational study, modelling species, and others. Matlab was used to model the predicted logK values for salicylic acid binding to copper at various ionic strengths. The species and parameters of all reactions were included to complete

this modelling process, and the logK values were determined. This measurement of logK values was useful to predict the change of logK based on changing the ionic strength. All examples of the Matlab codes were presented in appendices section from A1 to A4.

2.7 Experiments direction

Table 2 presents the overall experiments. There were three steps of the operations to test the change in the formation constant of copper-salicylic acid. The first procedure was determining the experiment between copper and salicylic acid without adding salt. Then, five experiments were designed with various ionic strengths. Lastly, five tests were designed with multiple concentrations of ASW. Each experiment was replicated three times (Table 2).

Table 2. The overall experimental work.

<i>Experiments</i>	Data Analysis
1) <i>Salicylic Acid</i>	<p>a) First experiment:</p> <ul style="list-style-type: none"> ➤ pH 8 ➤ 10 μM of Salicylic acid ➤ Titrate Cu: 0 \longrightarrow 316 μM ➤ Determine logK using RW. ➤ Determine theoretical logK by DH. ➤ Determine logK by interpolation of NIST data.
	<p>b) Second experiment:</p> <ul style="list-style-type: none"> ➤ Repeat section (a).

- Adding inert salt $\text{Na}_2\text{SO}_{4(s)}$ with ionic strengths of:

[0, 0.03, 0.15, 0.75, 1.2]

c) Third experiment:

- Repeat section (b).
- In artificial seawater condition with concentrations of [10%, 25%, 50%, 75% and 100%]

Chapter 3

Results and Discussion

3.1 Ryan-Weber curve prediction

Experiments had been conducted on the effect of various ionic strength on the formation constant of Cu-Sal, which used artificial seawater and Na_2SO_4 salt. The response of the fluorescence with each addition of copper in multiple concentrations of Na_2SO_4 (S) and artificial seawater at pH 8 can be found in both Fig. 5 and 6, respectively. For each point of the curves, it showed the replicate of each experiment 3 times and was used 10 μM of salicylic acid. For all concentrations of media, the fluorescence was quenched by each addition of copper, which indicates the binding between copper and salicylic acid. For these RW plots, the fluorescence is plotted as a function of the total copper added at the maximum ($\text{ex}= 282$, $\text{em}= 404.6$) of salicylic acid. Each fluorescent value was normalized fluorescence for that titration. Thus, all titrations start with a relative fluorescence value of 1.0. These fluorescence responses were fitted by using RW equation as described above in equation 9 of the fluorescence quenching section above.

Figure 5 presents five best fit lines. It illustrates the intensity of the fluorescence quenching versus the total copper with varying ionic strength in Na_2SO_4 (S) media. The initial red curve determines the steepness of the curve between copper and salicylic acid at zero ionic strength, which is influenced by the $\log K$ value. It shows the steepness curve between copper

and ligand compare to other curves as the fluorescence quenched differently by changing the ionic strength in the solution. The reason for this steep curve is because there is no effect of other ions in the solution or competing species, so the interaction between copper and salicylic acid is unimpeded. By increasing the ionic strength of the solution, as seen in Fig 5, the steepness of the curves is decreased because of the effect of ions in the solution. The ionic strength of 0.4 line is a smaller rate of change versus added copper and has the highest ionic strength. The high ionic strength means high concentrations of ions. When the concentrations of ions are increased in the solution, the ions will form the ionic atmosphere around the copper and salicylate ions. This ionic atmosphere will reduce the actual bonding between copper and salicylic acid. Thus, the fluorescence quenching is decreased by increasing the ionic strength while the greater changes were determined at lower ionic strength Fig. 5.

In addition, the intensity of the fluorescence quenching versus the total copper at varying ionic strength in artificial seawater media demonstrates in Fig. 6. The initial experiments of artificial seawater media were at 10% of seawater. This lower concentration of seawater has the steepest curve between copper and salicylic acid close to the initial data point for the media that does not contain any amount of salt. The curves show only slight differences at higher ionic strength. However, they are varied in $\log K$ predicted. This is because of the effect of the competing species and other ligand complexations that present in seawater. By increasing the concentrations of seawater, the steepness of the curve was decreased. Although the activity corrections for seawater were included in these fitting curves, there are no significant differences in the bending of the curve determined for copper bind to salicylic acid at higher salt concentrations. However, the change is still greater compared to lower salt concentration. The steepest curve was determined at zero ionic strength compared to other curves because there are

no other reactions influenced the binding between copper and salicylic acid, so the complex can form easily Fig. 6.

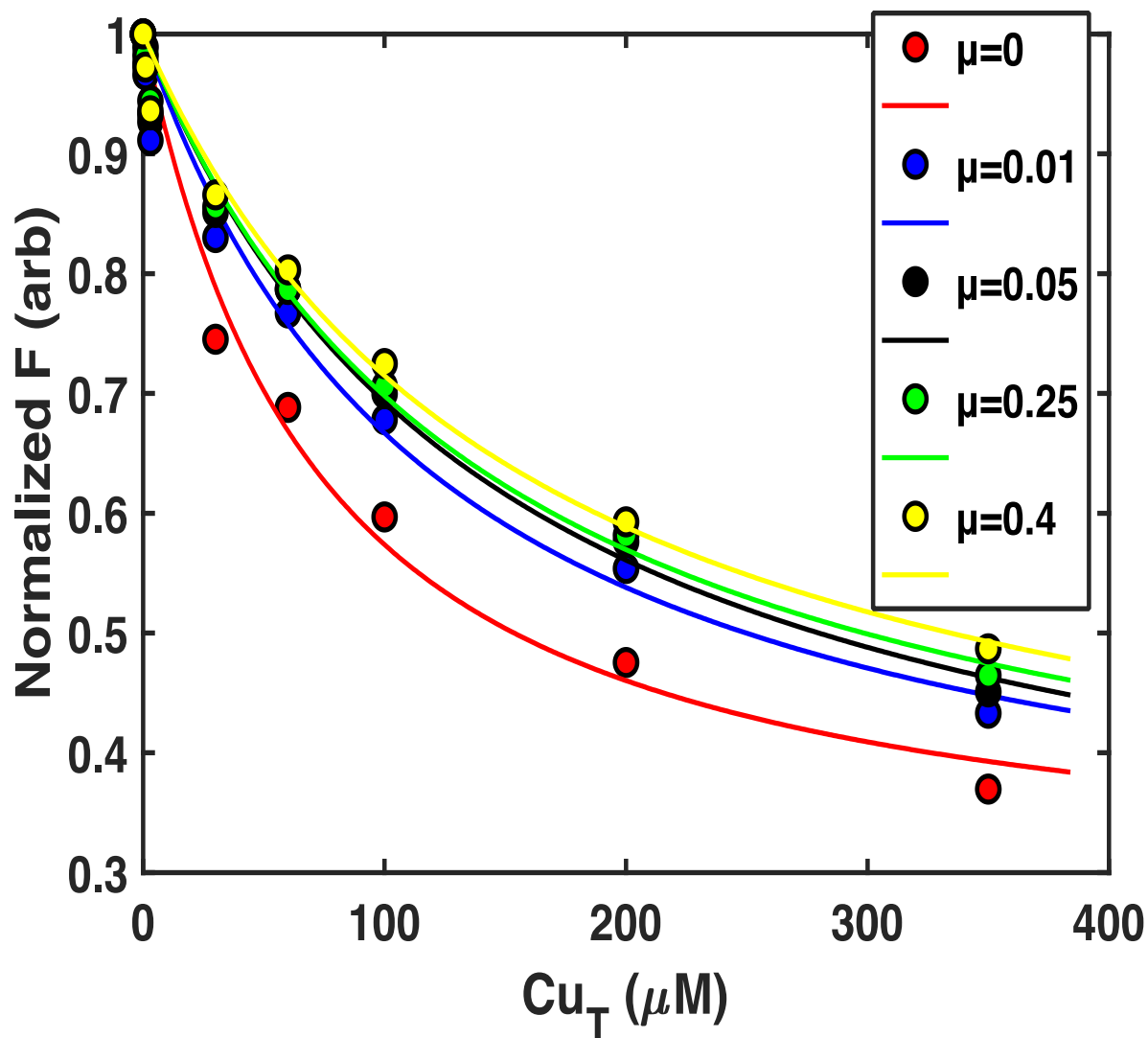


Figure 5. A decrease in the intensity of the normalized fluorescence quenching versus the total concentrations of copper with the varying ionic strength of Na_2SO_4 (s).

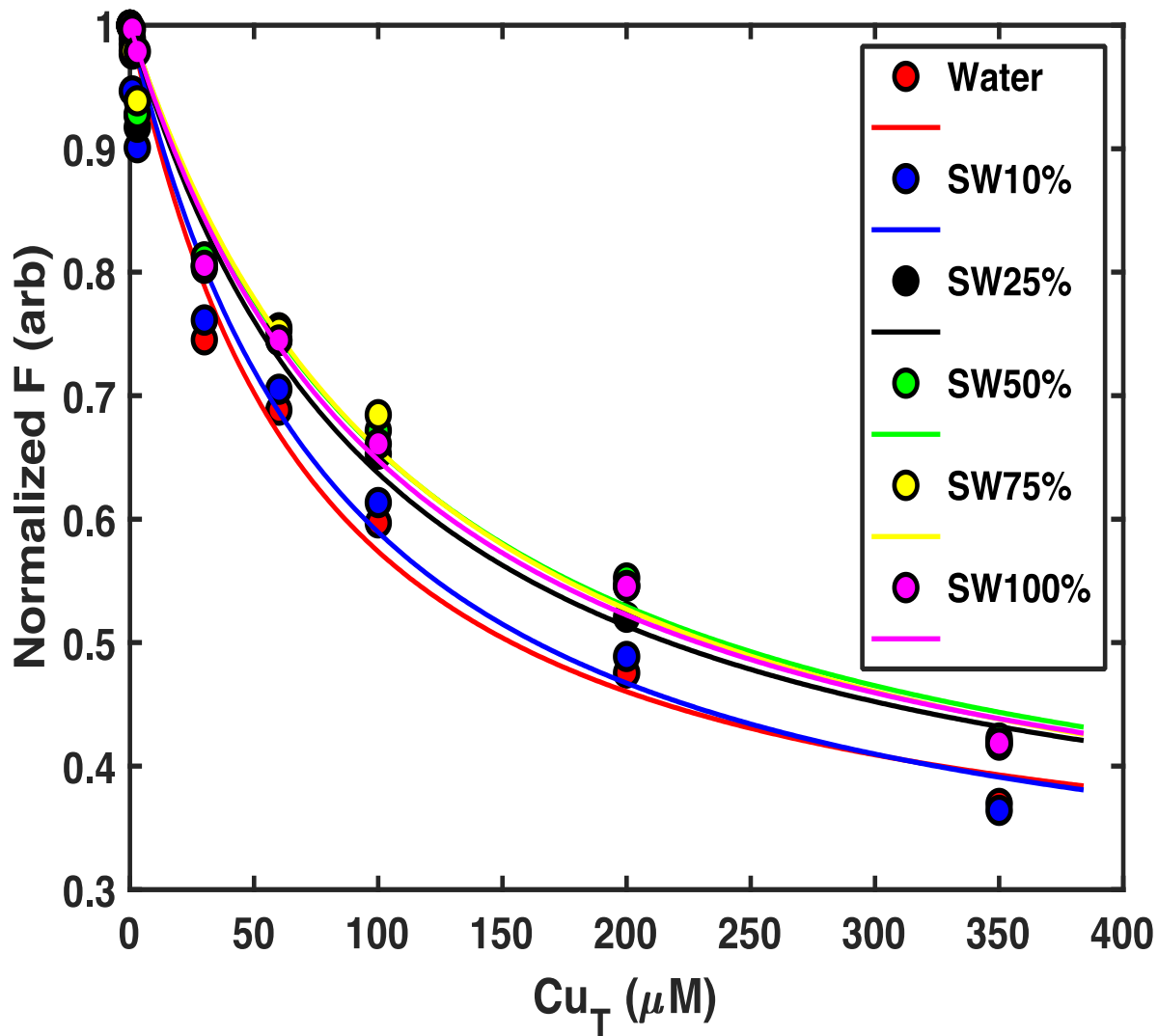


Figure 6. A decrease in the intensity of the fluorescence quenching versus the total concentrations of copper with the varying ionic strength of artificial seawater media.

3.2 logK values determination

The predicted logK values determined by fitting the RW equation for both types of media are shown in Fig. 7 and 8. The figures present the logK values versus ionic strength in both

media with the error bars (95% confidence interval) for the measured values. The equation of the error bars calculation is shown below:

$$\text{Confidence interval} = \pm \frac{ts}{\sqrt{n}} \quad (10)$$

t = student's t for 95% confidence and n-1 the degrees of freedom

s = standard deviation

n = number of values in average = 3

Moreover, the measured fluorescence quenching in the blue data points present the best fit of the logK values for copper binding to salicylic acid model versus the ionic strength for the measured values while the yellow data points indicate the NIST interpolations. Finally, the measured red lines were from the calculation of the theoretical activity correction using the extended DH equation. The predicted logK values for all media can be found in Table 3 for media that contains different concentrations of Na_2SO_4 (s) and Table 4 for media that contains various concentrations of artificial seawater.

3.2.1 Model ligand study in Na_2SO_4 (s)

The measured blue data points (Fig. 7) shows the direct measurements of the logK value for copper and salicylic acid, as determined by fluorescence quenching. These experiments were used $10 \mu\text{mol} \cdot \text{L}^{-1}$ of salicylic acid as a model ligand to bind with copper metal at pH 8. The ionic strength of the solutions was change by adding various concentrations of Na_2SO_4 (s), which affect the results of logK values. The logK value for the given reaction was 12.28 (NIST, 2010) for zero ionic strength; however, by increasing the ionic strength, the logK values were

decreased. This is because of the effect of the ionic atmosphere, which is affected by the concentrations and activity of ions. When the ionic strength increases in the solution, several events occur. One of these events is that cations and anions can bind to each other, such as binding between copper and salicylic acid. Also, the cation can be surrounded anions or vice versa to create an ionic atmosphere. The ionic atmosphere has a lower effective charge than the actual ion charge. As a result, there is less attraction between copper and salicylate and a reduced K_f value.

In addition, there is no significant difference predicted in the results of $\log K$ values for solutions that contain 0.75 and 1.2 ionic strength because of the high concentrations of ions.

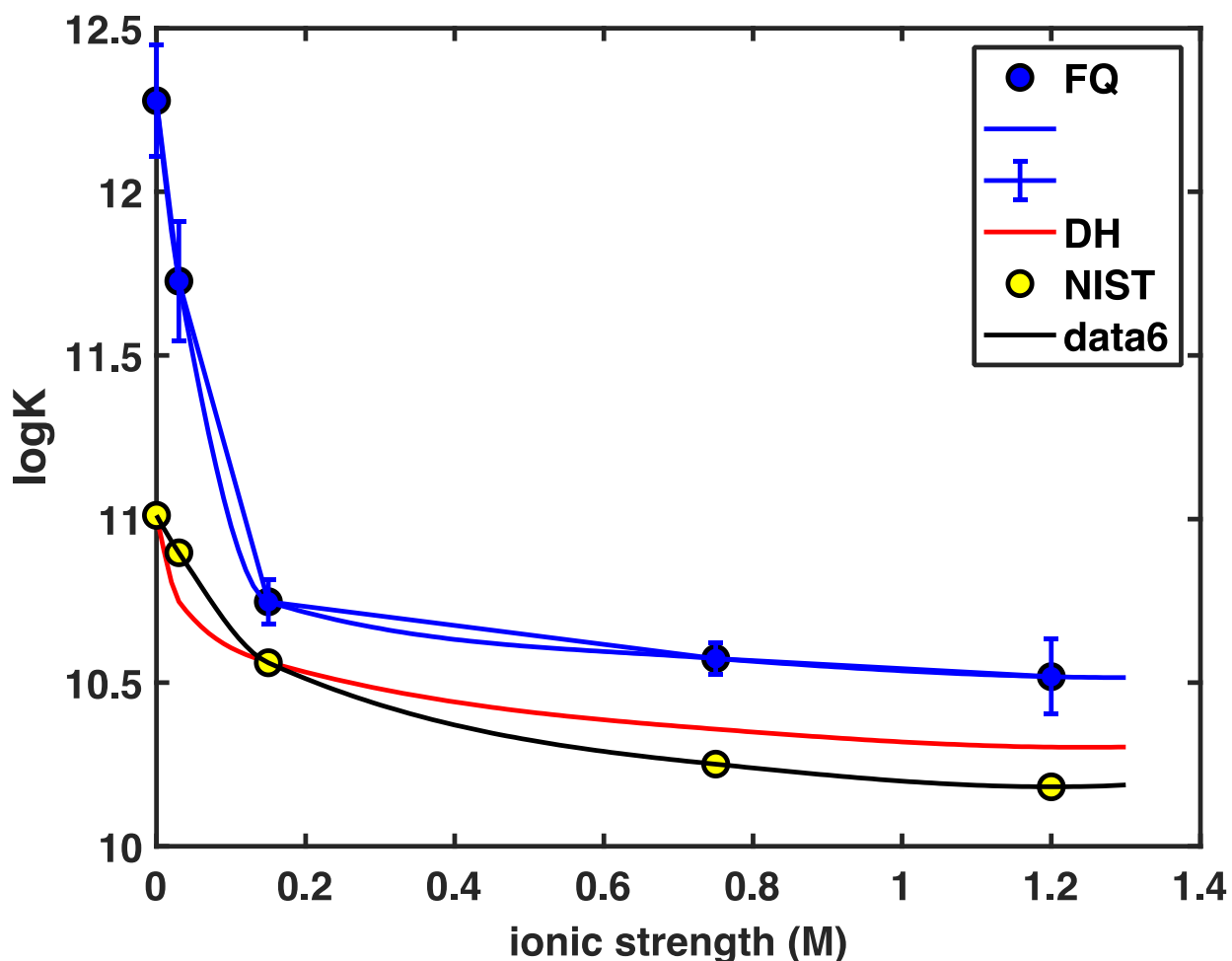


Figure 7. The determination of logK values versus ionic strength of copper binding salicylic acid as Model ligand study, NIST interpolation, and theoretical study in varying concentrations of Na_2SO_4 (S). Each logK values predicted was replicated three times, and the pH was 8.

Table 3. Predicted logK values of measured values from direct measurements, NIST interpolation, and extended DH equation in varying concentrations of Na_2SO_4 (S).

Ionic strength (M)	Direct measurement (logK)	NIST (logK)	DH (logK)
0	12.29	11.01	11.01
0.03	11.73	10.90	10.75
0.15	10.75	10.56	10.56
0.75	10.57	10.25	10.36
1.2	10.51	10.19	10.30

3.2.2 Comparison between NIST and DH in Na_2SO_4 (S)

In Fig 7 below, the measured values in yellow data points (Fig. 7) present the NIST interpolations. NIST has measured values that were calculated the formation constant of copper binding to salicylic acid at various ionic strength. These measured values were interpolated assuming that the logK values for any ionic strength between these measured values should be followed the interpolated line. Each of these measured values form NIST was titrated and measured experimentally again using the fluorescence quenching technique. Then, all of the

measured values were plotted using the RW equation to determine the logK values for each experiment. Thus, the results of the logK values determined versus ionic strength were interpolated to make a comparison between NIST and measured data interpolation. This interpolation is useful because this study does not measure every single ionic strength. The results of the comparison between the logK values of NIST and measured FQ show that the logK values predicted of FQ measurement are higher than the logK of NIST.

The theoretical study using the extended DH equation was determined in Fig. 7 as a red line. The initial point of this calculations was at zero ionic strength, and it has a logK value of 11.01. At zero ionic strength, the logK value for both theoretical and thermodynamic value are constant for the given reaction, but they are shown a slight difference by one order magnitude compared to the other points because of the effect of ions in the system. Moreover, in the initial point for the thermodynamic reaction, there is no any other competing species to make other reactions in the system; thus, the logK value determine only for ligand complexation that the study focused on. For the initial point of the theoretical prediction, it used the formation constant only that already predicted for the binding between copper and salicylic acid. There is a table present the formation constant for most known reactions.

In addition, in the theoretical calculations to determine the thermodynamic logK values, it used the formation constant, which is 11.01 for the binding between copper and salicylic acid. Then, this formation constant was multiplied with the calculated activity by using the extended DH equation, which is related to the ionic strength and concentrations. This multiplication of the formation constant and activity gave the corrected logK value. Although both the theoretical and thermodynamic logK values included corrections, there are negligible difference in the logK values predicted as stated above.

Furthermore, in a high concentration of the ionic strength, such 0.75 and 1.2, the logK values that predicted using the extended DH equation were between the logK values predicted for direct measurement and NIST interpolation. The reason of this varying is because the activity of ions is influenced the results. The changes of the curve are still much lower in higher concentrations.

As a comparison for the results of the titrations, the logK values determined from FQ were greater compared to the NIST and DH values. One of the possible reasons for this difference is because the FQ can be quenched in two ways, which are dynamically or statically as stated above in the method section. The dynamic quenching could contribute to the static quenching, which can influence the consequence of the logK values predicted. It is important to point out that is difficult to separate the static and dynamic portion of the quenching. Furthermore, the results of the extended DH agree with the NIST interpolation. This agreement shows that we can use the extended DH for BLM for estuary system. It is important to point out that the extended DH works with a small molecule only, such as salicylic acid, and not for humic and fulvic acid molecules because the activity coefficients for large molecules, which is important for DH calculations, are unknown (Harris, 2010).

3.2.3 Model ligand study in artificial seawater

Fig 8 demonstrates the determination of logK values versus ionic strength in artificial seawater. The blue data points present the direct measurements of copper binding to salicylic acid in the seawater condition at pH 8. The ionic strength was change in these experiments by using variable concentrations of artificial seawater. The initial point was measured at 10% of

artificial seawater. This percentage of the species for seawater was the lowest studied, and it has ionic strength of 0.069. The logK value predicted was 10.9. At the 25% and 50% of seawater, the logK values for both percentages were decreased and determined at 10.8. After the half concentration of seawater, the logK values started to increase again. The reason for this increase could be the effect of the speciation in seawater. There are more complicated reactions present in seawater compare to the media that contained Na_2SO_4 (s). The measured logK values from NIST, as well as the extended DH calculation, do not show such an increase. This increase was not observed in Na_2SO_4 solution.

3.2.4 Compare the theoretical study with artificial seawater

The red line in Fig. 8 illustrates the theoretical calculation using the DH equation the same one that used in Fig. 7. Also, the NIST was predicted similar to the Na_2SO_4 media graph (Fig.7). It was expected to see less quenching in high concentrations of seawater. The logK values were predicted much higher in the direct measurements compare to the DH calculation and NIST interpolation. These results mean that the direct fluorescence measurements do not match with other predicted logK values method in seawater. They were off by an order magnitude.

In addition, fluorescence quenching has some assumptions. One of these assumptions assumes the response of the fluorescence is linear with the concentrations (D. S. Smith & Kramer, 2000). The second assumption is that this quenching of the fluorescence is static. It assumes that a complex for salicylic acid binding to copper is less fluorescent than the original

molecule, which is present as a ground-state complex, so the fluorescence goes down. Compared to the direct measurements predicted, the results show more fluorescent than what is expected. The reason for increased fluorescence could be related to the effect of other species present in seawater that are not taken into account. Even though the seawater species, which are present in the tableau, were considered to determine the logK values for the reaction of interest (Cu-Sal), these results stated that the fluorescence measurements do not work well in sea water media.

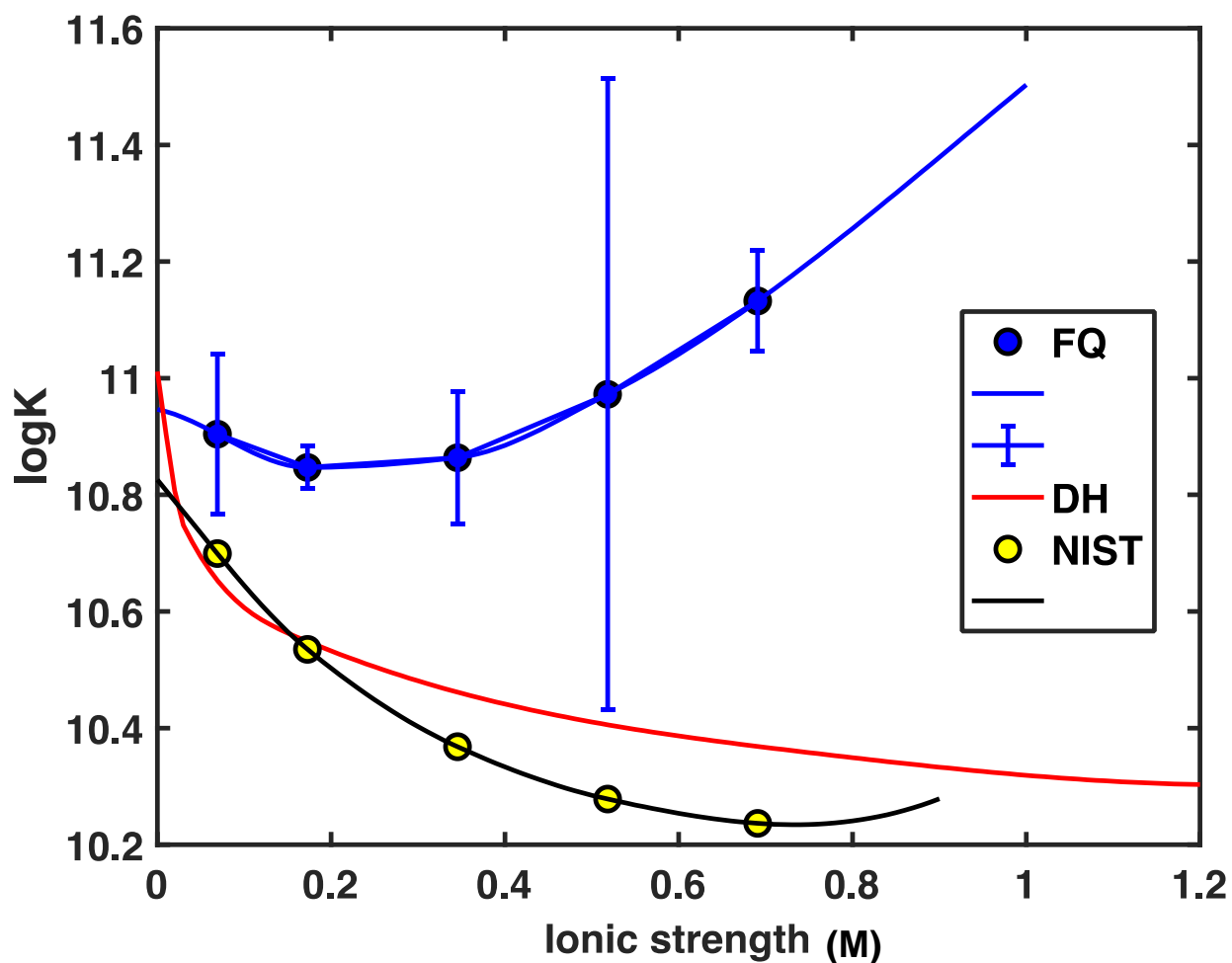


Figure 8. The determination of logK values versus ionic strength of copper binding salicylic acid as model ligand study, NIST interpolation, and theoretical study in varying

concentrations of artificial seawater. Each logK values predicted was replicated three times, and the pH was 8.

Table 4. Predicted logK values as direct measurements, NIST interpolation, and extended DH equation in varying concentrations of artificial seawater.

Ionic strength and percentage in seawater		Direct measurement (logK)	NIST (logK)
Ionic strength (M)	Percentage		
0.07	10%	10.90	10.70
0.17	25%	10.85	10.54
0.35	50%	10.86	10.37
0.52	75%	10.97	10.28
0.70	100%	11.13	10.24

3.2.5 LogK values for speciation in seawater

Fig. 9 illustrates the determination of logK values versus ionic strength for inorganic side reactions see (Tableau, appendices A1) present in seawater. The measured blue data points in figure 9 present the NIST measured values while the lines present the extended DH calculations. For most reactions, the extended DH calculations have a better agreement compared to NIST measured values. However, some of the reactions are totally disagreed such as H_2O and CuOH^+ , and this disagreement could be related to the effect of hydroxide ion. The hydroxide ion is capable of forming a strong hydrogen bond in water, which could result in an increase in the stability of hydroxide ion and lower the logK values experimentally. Another reason could be the

high concentrations of ions because the extended DH equation is intended for low ionic strength (Harris, 2010). There are other equations that could be used to calculate the activity, such as Davies equation. The Davies equation is an extension of the DH theory to measure the activity coefficient for electrolyte solution in a high concentration at 25°C. Davies equation in equation (10) is:

$$-\log f_{\pm} = .5 z_1 z_2 \left(\frac{\sqrt{I}}{1+\sqrt{I}} - .30 I \right) \quad (10)$$

where f_{\pm} is the activity coefficient in molal unit, and both z_1 and z_2 are the charges. I is the ionic strength. Furthermore, in equation (10), when the ionic strength tends to be zero, the extended DH equation should be used at the low concentration 0.1 M. In addition, Davies equation is worthless for ionic strength greater than 0.3 M. For solutions with ionic strength above 0.1 there is Pitzer equations usually used for more complicated solutions. Pitzer equations are in equation 11 and 12, respectively. These equations work well with higher concentrations until about 6 mol.kg⁻¹.

$$\ln \gamma_{\pm}^{\pm} = \frac{p \ln \gamma_M + q \ln \gamma_X}{p+q} \quad (11)$$

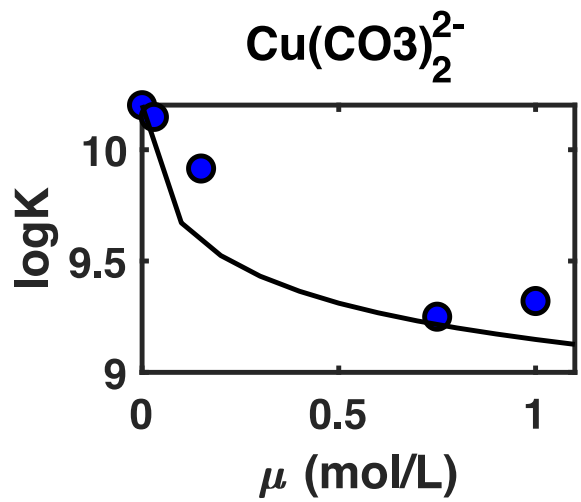
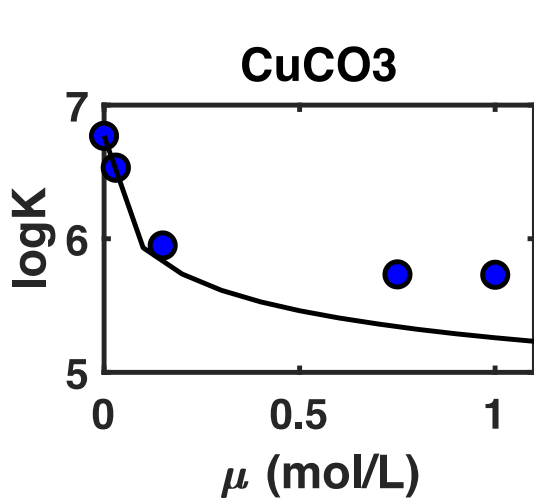
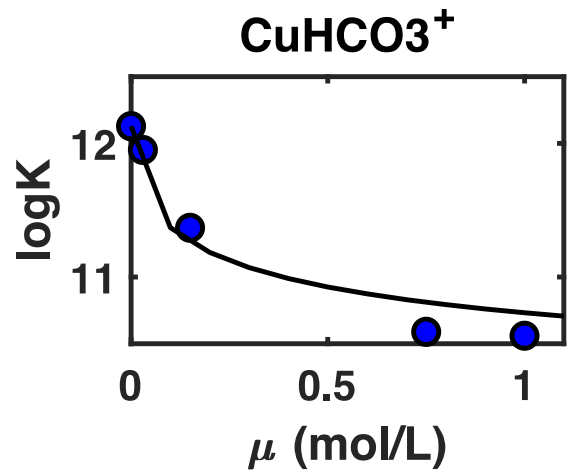
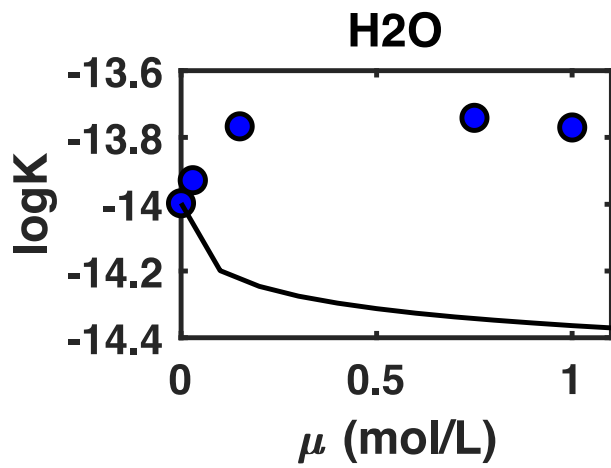
$$\ln \gamma_{\pm}^{\pm} = (z^+ z^-) \mathcal{F}^{\gamma} + m \left(\frac{2pq}{p+q} \right) B_{MX}^{\gamma} + m^2 \left[2 \frac{(pq)^{\frac{3}{2}}}{p+q} \right] C_{MX}^{\gamma} \quad (12)$$

$\ln \gamma_{\pm}^{\pm}$ = activity coefficient

$z^+ z^-$ = charge

\mathcal{F}^{γ} , m , p , q , B_{MX}^{γ} , C_{MX}^{γ} = calibration parameters

As a result of the calculation using the extended DH equation for the inorganic species, it shows a good agreement between the results of NIST interpolation and the extended DH equation.



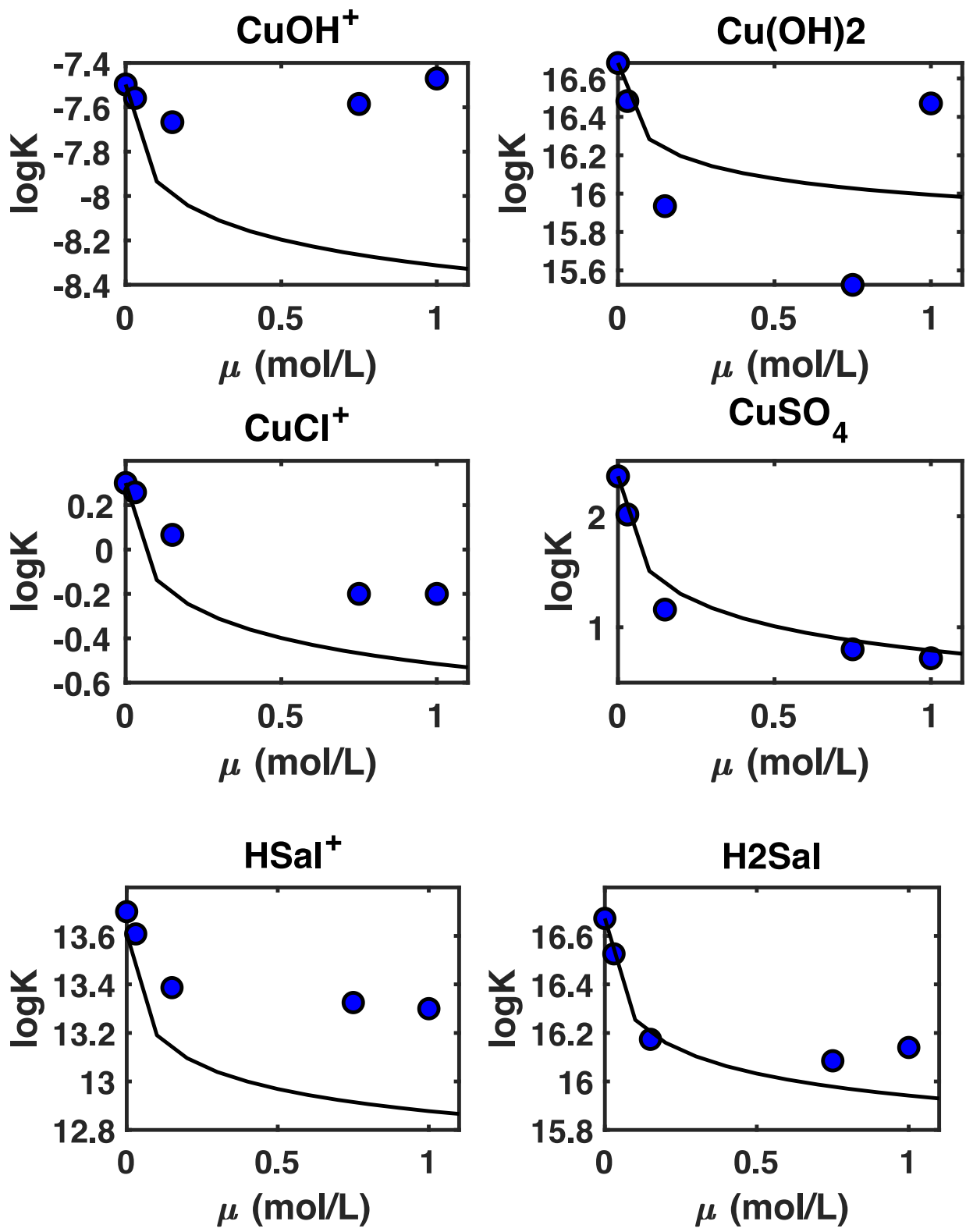


Figure 9. The determination of logK values versus ionic strength of some reactions for speciations present in seawater using extended DH equation.

3.2.6 Relationship between logK values

Fig. 10 determines a comparison of logK values between DH and direct measurements compared to NIST as one-to-one (1:1) line. The graphs show the predicted logK values for both measured (as blue data points for Na₂SO₄ and black data point for ASW solutions) and DH (as red data points) values versus logK for NIST. The NIST has certified accepted values, so it explains the quality of using FQ and DH methods to determine logK at different ionic strength compared to NIST values. The extended DH works well; however, the measured values using FQ is not very well. They do tend to work better at the higher ionic strength when K values are lower, as seen in the previous figure (Fig.7), the lower logK values at higher ionic strength. It is off by order magnitude but that is not very far from other methods determined the logK; however, in a very dilute sample, it would show greater disagreements.

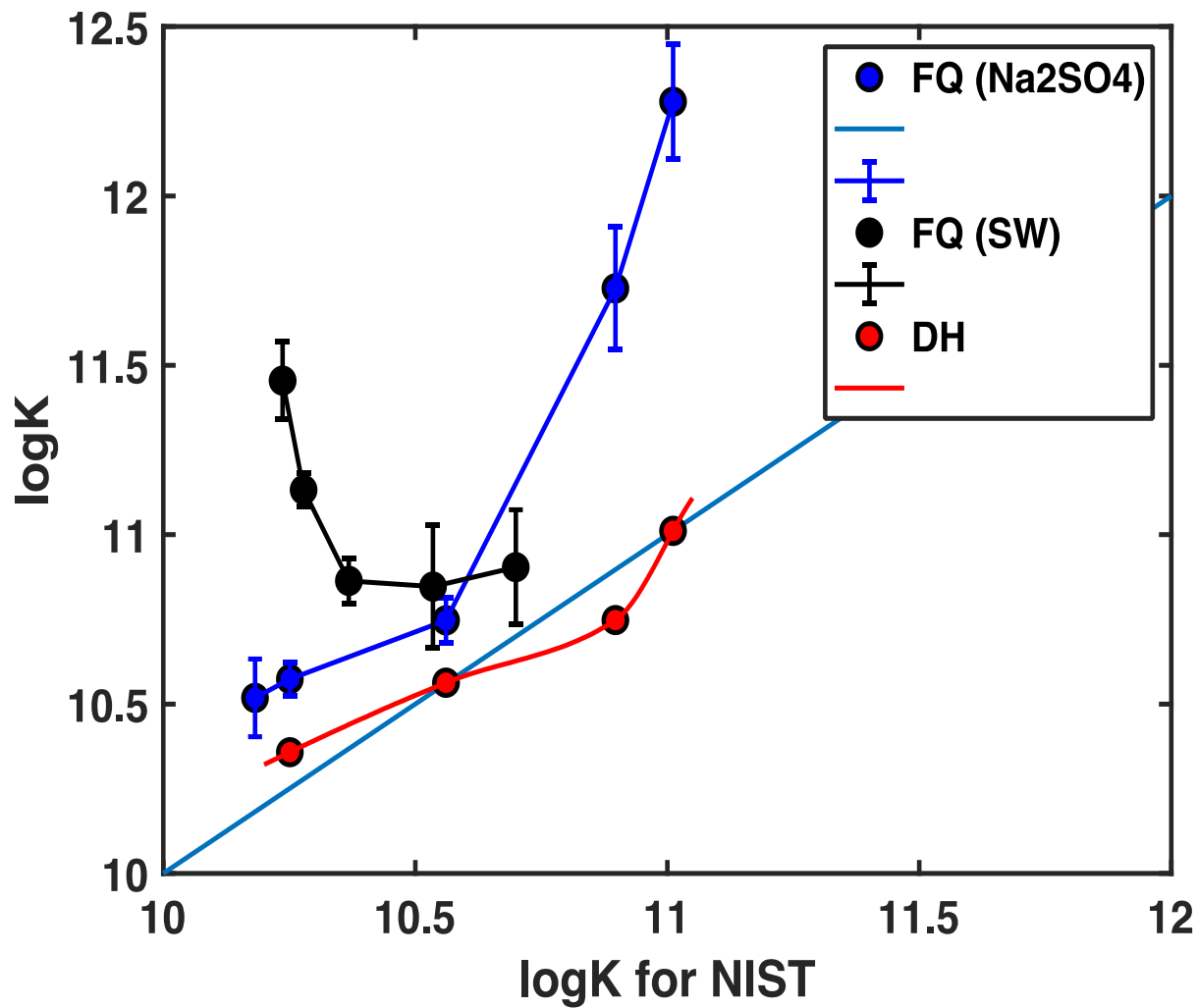


Figure 10. The determination of logK values for NIST interpolation versus the predicted logK values for the direct measurement and extended DH calculations in $\text{Na}_2\text{SO}_4(\text{s})$ and ASW solutions.

Chapter 4

Conclusion and Future Work

4.1 Summary of research

In summary, impacts of multiple ionic strength on copper binding to salicylic acid has tested in this study, and how they affect the chemical side of the BLM in estuary systems by using fluorescence quenching. The predicted logK values for the direct measurements were off by an order of magnitude factor 10 compared to the NIST interpolation and DH equation. This study found that the fluorescence quenching method is not a good tool to use for testing the copper speciations in the estuarine environment because it does not agree with NIST measured values. There could be something fundamentally wrong with the fluorescence method, in particular, the RW method assumes static quenching. If there was additional dynamic quenching, then the logK values tend to be overestimated.

Furthermore, this study show agreement between the NIST values and DH calculation. The DH calculations includes the ionic strength and the ion size for each species in the system. There is a table present the ion size for most small known molecules (Harris, 2010). The results show that DH works well for small molecules. For a large molecule such as NOM, it could not be easily calculated using the DH equation. It remains necessary to develop methods for Cu-NOM speciation studies in salt water.

Accordingly, neither the DH can be used for a large molecule nor the results of the fluorescence work well, so these methods are not a promising tool to test the effect of various ionic strength on the bioavailability of copper in a large molecule.

4.2 Future work

As a future work for this research, it could be used a different method such as Ion-Selective Electrode (ISE) to test the bioavailability of copper in the varying ionic strength using salicylic acid as a ligand. If the results of the ISE work well and match with the NIST interpolation and DH calculations, another ligand can be used to bind with copper such as NOM. Then, the change in the results can be compared to each other. However, it should be considered that the DH could not be used for a large molecule such as NOM.

Appendices

A1. Tableau of the speciation in seawater

%H	Cu	CO3	Cl	L	SO4	logK	species
1	0	0	0	0	0	0	{ 'H' }
0	1	0	0	0	0	0	{ 'Cu' }
0	0	1	0	0	0	0	{ 'CO3' }
0	0	0	1	0	0	0	{ 'Cl' }
0	0	0	0	1	0	0	{ 'L' }
0	0	0	0	0	1	0	{ 'SO4' }
-1	0	0	0	0	0	logKw	{ 'OH' }
-1	1	0	0	0	0	logKh1	{ 'CuOH' }
-2	1	0	0	0	0	logBh2	{ 'CuOH2' }
-3	1	0	0	0	0	logBh3	{ 'CuOH3' }
-4	1	0	0	0	0	logBh4	{ 'CuOH4' }
-2	2	0	0	0	0	logBh22	{ 'Cu2OH2' }
1	0	1	0	0	0	H2CO3pKa2	{ 'HCO3' }
2	0	1	0	0	0	H2CO3pBa2	{ 'H2CO3' }
0	1	1	0	0	0	logKCcuCO3	{ 'CuCO3' }
0	1	2	0	0	0	logKCcuCO32	{ 'CuCO32' }
1	1	1	0	0	0	logKCcuHCO3	{ 'CuHCO3' }
0	1	0	1	0	0	logKCcuCl	{ 'CuCl' }
0	1	0	0	1	0	logKCcuL	{ 'CuL' }
1	0	0	0	1	0	H2LpKa2	{ 'HL' }
2	0	0	0	1	0	H2LpBa2	{ 'H2L' }
0	1	0	0	0	1	logKCcuSO4	{ 'CuSO4' }
0	1	0	0	2	0	logBCcuL2	{ 'CuL2' }
1	1	0	0	1	0	logKCcuHL	{ 'CuHL' }

A2. Matlab example code of DH calculations

```
% x-DH equation
figure(1); subplot(221); clf
subplot(222); clf
subplot(223); clf
subplot(224); clf

%reaction Cu+OH=CuOH -----
-----

I=[0 0.03 0.15 0.75 1];
logK=[-7.4970 -7.5582 -7.6668 -7.5848 -7.4700];

K0=10^-7.4970;
mu=[0:0.1:1.1];
loggamma=@(z,mu,a) (-0.51*z^2*sqrt(mu))/(1+(a*sqrt(mu)/305));
%loggamma=@(z,mu,a) (-0.51*z^2*sqrt(mu)); % debye huckel
for i=1:size(mu,2)

    loggammaCu=loggamma(2,mu(i),600); gammaCu=10^loggammaCu;
    loggammaCuOH=loggamma(1,mu(i),900); gammaCuOH=10^loggammaCuOH;
    loggammaOH=loggamma(-1,mu(i),300); gammaOH=10^loggammaOH;

    Kconc=K0*(gammaCu*gammaOH)/gammaCuOH;
    logKconc(i)=log10(Kconc);
end

figure(1); subplot(221)
plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(mu,logKconc,'k','linewidth',2)
set(gca,'linewidth',2,'fontsize',12)
xlabel('\mu (mol/L)'); ylabel('logK')
title('CuOH^+')

%reaction Cu+2OH=Cu(OH)2 -----
-----

I=[0 0.03 0.15 0.75 1];
logK=[16.6810 16.4823 15.9349 15.5245 16.4700];

K0=10^16.6810;
mu=[0:0.1:1.1];
loggamma=@(z,mu,a) (-0.51*z^2*sqrt(mu))/(1+(a*sqrt(mu)/305));
%loggamma=@(z,mu,a) (-0.51*z^2*sqrt(mu)); % debye huckel
for i=1:size(mu,2)

    loggammaCu=loggamma(2,mu(i),600); gammaCu=10^loggammaCu;
```

```

loggammaCuOH2=loggamma(0,mu(i),1250); gammaCuOH=10^loggammaCu;
loggammaOH=loggamma(-1,mu(i),350); gammaOH=10^loggammaCu;

Kconc=K0*((gammaCu*gammaOH)/gammaCuOH);
logKconc(i)=log10(Kconc);
end

figure(1); subplot(222)
plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(mu,logKconc,'k','linewidth',2)
set(gca,'linewidth',2,'fontsize',12)
xlabel('\mu (mol/L)'); ylabel('logK')
title('Cu(OH)2')

% Cu+ Cl=CuCl -----

I=[0 0.03 0.15 0.75 1];
logK=[0.3 0.2577 0.0660 -0.2 -0.2];

K0=10^0.3;
mu=[0:0.1:1.1];
loggamma=@(z,mu,a) (-0.51*z^2*sqrt(mu))/(1+(a*sqrt(mu)/305));
%loggamma=@(z,mu,a) (-0.51*z^2*sqrt(mu)); % debye huckel
for i=1:size(mu,2)

    loggammaCu=loggamma(2,mu(i),600); gammaCu=10^loggammaCu;
    loggammaCuCl=loggamma(1,mu(i),900); gammaCuCl=10^loggammaCuCl;
    loggammaCl=loggamma(-1,mu(i),300); gammaCl=10^loggammaCl;

    Kconc=K0*((gammaCu*gammaCl)/gammaCuCl);
    logKconc(i)=log10(Kconc);
end

figure(1); subplot(223)
plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(mu,logKconc,'k','linewidth',2)
set(gca,'linewidth',2,'fontsize',12)
xlabel('\mu (mol/L)'); ylabel('logK')
title('CuCl^+')

% reaction SO4+Cu=CuSO4 -----
---
```

```

K0=10^2.36;
mu=[0:0.1:1.1];
loggamma=@(z,mu,a) (-0.51*z^2*sqrt(mu))/(1+(a*sqrt(mu)/305));
%loggamma=@(z,mu,a) (-0.51*z^2*sqrt(mu)); % debye huckel
for i=1:size(mu,2)

    loggammaCu=loggamma(2,mu(i),600); gammaCu=10^loggammaCu;
    gammaCuSO4=1; % neutral
    loggammaSO4=loggamma(-2,mu(i),400); gammaSO4=10^loggammaSO4;
    Kconc=K0*((gammaCu*gammaSO4)/gammaCuSO4);
    logKconc(i)=log10(Kconc);
end

figure(1); subplot(224)
plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(mu,logKconc,'k','linewidth',2)
set(gca,'linewidth',2,'fontsize',12)
xlabel('\mu (mol/L)'); ylabel('logK')
title('CuSO_4')

```


A3. Matlab example of the NIST interpolated measured vales code

```
% find seawater appropriate logK values relevant to Cu speciation
% with salicylic acid as an added ligand
% just use 25 degrees C values
```

```
figure(1); clf
% ionic strength for interpolation
```

```
I0pt6=0;
```

```
%%
% OH
% reaction H+OH=H2O
```

```
I=[0 0.1 0.5 0.7 1];
logK=[13.997 13.78 13.73 13.75 13.77];
Iinterp=0:0.1:1;
YI = interp1(I,logK,Iinterp,'cubic');
```

```
logKcorr = interp1(I,logK,I0pt6,'cubic'); logKw=-1*logKcorr;
logKcorrtxt=num2str(logKcorr,5);
```

```
plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)
```

```
logKw=logKw
print Kwcorrection.eps -depsc2
```

```
%%
```

```
% CuOH
% reaction Cu+OH=CuOH
%%
```

```
figure(1); clf
```

```
I=[0 0.1 0.5 0.7 1];
logK=[6.5 6.1 6.1 6.2 6.3];
```

```

Iinterp=0:0.1:1;
YI = interp1(I,logK,Iinterp,'cubic');

%I0pt6=1.05;
logKcorr = interp1(I,logK,I0pt6,'cubic'); logKOH1=logKcorr;
logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)

%Cu+H2O=CuOH for tableau
logKh1=logKOH1+logKw
%%

% H2CO3
% reaction CO3+H=HCO3
%%

figure(1); clf

I=[0 0.1 0.5 0.7 1.0];
logK=[10.329 9.90 9.61 9.53 9.52];
Iinterp=0:0.1:1;
YI = interp1(I,logK,Iinterp,'cubic');

logKcorr = interp1(I,logK,I0pt6,'cubic'); logKa2=logKcorr;
logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)
%H+CO3=HCO3 for tableau
logKa2=logKa2

print H2CO3pKa2.eps -depsc2

```

```

%%

% H2CO3
% reaction HCO3+H=H2CO3
%%

figure(1); clf

I=[0 0.1 0.5 0.7 1.0];
logK=[6.352 6.13 6.00 5.97 6.95];
Iinterp=0:0.1:1;
YI = interp1(I,logK,Iinterp,'cubic');

logKcorr = interp1(I,logK,I0pt6,'cubic'); logBa2=logKcorr+logKa2;
logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)

print H2CO3pKa1.eps -depsc2

%2H+CO3=H2CO3 for tableau
logBa2=logBa2
%%
% H2CO3
% reaction Cu+CO3=CuCO3
%%

figure(1); clf

I=[0 0.1 0.7 1];
logK=[6.77 6 5.73 5.73];
Iinterp=0:0.1:1;
YI = interp1(I,logK,Iinterp,'cubic');

logKcorr = interp1(I,logK,I0pt6,'cubic'); logKCuCO3=logKcorr;
logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)

```

```

isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)

%Cu+CO3=CuCO3 for tableau
logKCuCO3=logKCuCO3
%%

% H2CO3
% reaction Cu+2CO3=Cu(CO3)2
%%
figure(1); clf

I=[0 0.1 0.7 1];
logK=[10.2 10 9.23 9.32];
Iinterp=0:0.1:1;
YI = interp1(I,logK,Iinterp,'cubic');

logKcorr = interp1(I,logK,I0pt6,'cubic'); logKCuCO32=logKcorr;
logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)
%Cu+2CO3=Cu(CO3)2 for tableau
logKCuCO32=logKCuCO32
%%

% H2CO3
% reaction Cu+HCO3=CuHCO3
%%
figure(1); clf

I=[0 0.7 1];
logK=[1.8 1.03 1.04];
Iinterp=0:0.1:1;
YI = interp1(I,logK,Iinterp,'cubic');

logKcorr = interp1(I,logK,I0pt6,'cubic'); logKCuHCO3=logKcorr;

```

```

logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)
%Cu+CO3+H=CuHCO3 for tableau
logKCuHCO3=logKCuHCO3+logKa2
%%

% CuCl
% reaction Cu+Cl=CuCl
%%
figure(1); clf

I=[0 0.5 0.7 1.0];
logK=[0.3 -0.2 -0.2 -0.2];
Iinterp=0:0.1:1;
YI = interp1(I,logK,Iinterp,'cubic');

logKcorr = interp1(I,logK,I0pt6,'cubic'); logKCuCl=logKcorr;
logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)
%Cu+Cl=CuCl for tableau
logKCuCl=logKCuCl
%%

% SO4
% reaction SO4+H=HSO4
%%
figure(1); clf

I=[0 0.1 0.5 1];

```

```

logK=[1.99 1.54 1.27 1.08];
Iinterp=0:0.1:1;
YI = interp1(I,logK,Iinterp,'cubic');

logKcorr = interp1(I,logK,I0pt6,'cubic'); logKHSO4=logKcorr;
logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)

%H+SO4=HSO4 for tableau
logKHSO4=logKHSO4
%%

% SO4
% reaction SO4+Cu=CuSO4
%%
figure(1); clf

I=[0 0.1 0.5 1];
logK=[2.36 1.26 0.85 0.72];
Iinterp=0:0.1:1;
YI = interp1(I,logK,Iinterp,'cubic');

logKcorr = interp1(I,logK,I0pt6,'cubic'); logKCuSO4=logKcorr;
logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)

%Cu+SO4=CuSO4 for tableau
logKCuSO4=logKCuSO4
%%

```

```

% Salicylic acid
% reaction H+Sal=HSal
%%
figure(1); clf

I=[0 0.1 1.0 3.0];
logK=[13.7 13.4 13.3 13.1];
Iinterp=0:0.1:3.1;
YI = interp1(I,logK,Iinterp,'cubic');

logKcorr = interp1(I,logK,I0pt6,'cubic'); logKHSal=logKcorr;
logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)

%H+Sal=HSal for tableau
logKHSal=logKHSal
%%

% Salicylic acid
% reaction H+HSal=H2Sal
%%
figure(1); clf

I=[0 0.1 0.5 1.0 3.0];
logK=[2.972 2.80 2.75 2.84 3.16];
Iinterp=0:0.1:3.1;
YI = interp1(I,logK,Iinterp,'cubic');

logKcorr = interp1(I,logK,I0pt6,'cubic'); logKH2Sal=logKcorr;
logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)

```

```

ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)

%2H+Sal=H2Sal for tableau
logKH2Sal=logKH2Sal+logKHSal
%%

% Sal
% reaction Cu+HSal=CuSal+H
%%
figure(1); clf

I=[0.1 0.5 1.0];
logK=[-2.78 -3.05 -3.11];
Iinterp=0:0.1:1;
YI = interp1(I,logK,Iinterp,'cubic');

logKcorr = interp1(I,logK,I0pt6,'cubic'); logKCuSal=logKcorr;
logKcorrtxt=num2str(logKcorr,5);

plot(I,logK,'ko','markersize',10,'markerfacecolor','b')
hold on
plot(Iinterp,YI,'k','linewidth',2)
set(gca,'fontsize',12,'linewidth',2)
isstr=num2str(I0pt6);
txt=['ionic strength (M), at ',isstr,'M logK = ',logKcorrtxt];
xlabel(txt,'fontsize',12)
ylabel('logK')
plot([I0pt6 I0pt6],[min(logK)-0.005*(min(logK)) logKcorr],'b--',
'linewidth',2)
plot([0 I0pt6],[logKcorr logKcorr],'b--','linewidth',2)

%Cu+Sal=CuSal for tableau
logKCuSal=logKCuSal+logKHSal

% ignore 2ligand 1 metal reaction.

```


A4. Matlab example code of RW equation

```
function name_0MNa2SO4

% first load and plot the data

[CuT,Fmeas]=getdata;

figure(1);
plot(CuT*1e6,Fmeas,'ko','markersize',10,'markerfacecolor','r')
set(gca,'linewidth',2,'fontsize',12); xlabel('Cu_T (\muM)'); ylabel('F
(arb)');

% calculate the fluorescence as a function of the logK for Cu+L=CuL
% and fraction less efficeint CuL fluorescence is (efficeincey factor
EF)
% LT is fixed and so is pH
% estimate kHL for fluorescence from the first point

#####
logK=10.2; EF=0.2; %change if necessary to get a good initial guess
#####

logEF=log10(EF); p=[logK logEF]; LT=10e-6; pH=8;
CuTplot=[1e-8:1e-6:max(CuT)*1.1]; kHL=Fmeas(1)/LT;

flag=1; % return F if flag =1. return error if flag =0;

F=returnForerr(p,CuTplot,LT,pH,kHL,Fmeas,flag);

%figure(1); hold on
%plot(CuTplot*1e6,F,'b--','linewidth',2)
%pause
% now fitting -----

pguess=[logK logEF];
options = optimset(@fminsearch);
options = optimset(options,'Display','iter','TolFun',1e-4,'TolX',1e-
4,'MaxFunEvals',1000);

flag=0; % return error
%tst=returnForerr(pguess,CuT,LT,pH,kHL,Fmeas,flag)
%pause

f = @(p) returnForerr(p,CuT,LT,pH,kHL,Fmeas,flag);
[pbest]=fminsearch(f,pguess,options)

% now plot best fit
flag=1;
```

```

Fbest=returnForerr(pbest,CuTplot,LT,pH,kHL,Fmeas,flag);

figure(1); hold on
plot(CuTplot*1e6,Fbest,'r','linewidth',2)
end

function II=returnForerr(p,CuT,LT,pH,kHL,Fmeas,flag)

logK=p(1); EF=10^p(2); kCuL=EF*kHL;

if flag==1 % solve for fluorescence
    %solve for the speciation
    [HL,CuL]=CuLspeciation(CuT,pH,logK,LT);
    %solve for fluorescence
    F=kHL*HL+kCuL*CuL;
    II=F;
end

if flag==0 % solve for error
    %solve for the speciation
    [HL,CuL]=CuLspeciation(CuT,pH,logK,LT);
    %solve for fluorescence
    Fcalc=kHL*HL+kCuL*CuL;
    residuals=Fcalc-Fmeas;
    err=log10(sum(residuals.^2));
    II=err;
end

end

function [HL,CuL]=CuLspeciation(CuT,pH,logK,LT)

#####

% seawater concentrations (100%)
ClT=1e-16;
CT=1e-16;
SO4T=1;

#####

for i=1:size(CuT,2)

[concs,masserr,SOLUTIONNAMES]=SOLVE_TABLEAU(pH,CuT(i),CT,SO4T,ClT,LT,logK);

c=0;
for k=1:size(SOLUTIONNAMES,1)
    txt=[SOLUTIONNAMES(k,:), '(i)=concs(k)'];

```

```

        eval(txt)
        c=c+1;
    end

end

end

function
[concs,masserr,SOLUTIONNAMES]=SOLVE_TABLEAU(pH,CuT,CT,SO4T,ClT,LT,logK
)

global Asolution Ksolution T TYPX

[KSOLUTION,ASOLUTION,SOLUTIONNAMES]=get_equilib_defn(logK);

% adjust for fixed pH
[Ksolution,Asolution]=get_equilib_fixed_pH(KSOLUTION,ASOLUTION,pH);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%TOTAL VECTOR
T=[CuT; CT; ClT; LT; SO4T];

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% solve solution only problem %%%%%%%%%%

X=T./10; [xguess,masserr,J,C] = nl_massbalancernsolid_NR(X);
concs=C;

end

% SUBFUNCTIONS
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

function [KSOLUTION,ASOLUTION,SOLUTIONNAMES] = get_equilib_defn(logK)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% from NIST K values. adjusted using interpolation mfile
% for specific ionic strength. and entered manually here

logKw= -13.9970;
logKh1= -7.4970;
H2CO3pBa2= 16.6810;
H2CO3pKa2= 10.3290;
logKCuCO3= 6.7700;
logKCuCO32= 10.2000;
logKCuHCO3= 12.1290;
logKCuCl= 0.3000;
logKCuL=logK; %this is our unknown we are fitting
H2LpBa2= 16.6720;
H2LpKa2= 13.7000;

```

```

logKCUSO4=    2.3600;

#####

logBh2=-14.8; % use fixed value. negligible species
logBh3=-27.2; % use fixed value. negligible species
logBh4=-40.4; % use fixed value. negligible species
logBh22=-10.98; % use fixed value. negligible species
logBCuL2=18.54; % use fixed value. negligible species
logKCuHL=15.24; % from that 1975 paper. negligible species.

Tableau=[...
%H      Cu      CO3      Cl      L      SO4      logK      species
1       0       0       0       0       0       0       {'H'}
0       1       0       0       0       0       0       {'Cu'}
0       0       1       0       0       0       0       {'CO3'}
0       0       0       1       0       0       0       {'Cl'}
0       0       0       0       1       0       0       {'L'}
0       0       0       0       0       1       0       {'SO4'}
-1      0       0       0       0       0       logKw    {'OH'}
-1      1       0       0       0       0       logKh1   {'CuOH'}
-2      1       0       0       0       0       logBh2   {'CuOH2'}
-3      1       0       0       0       0       logBh3   {'CuOH3'}
-4      1       0       0       0       0       logBh4   {'CuOH4'}
-2      2       0       0       0       0       logBh22  {'Cu2OH2  '}
%make name longer so solid and solution dimensions match when put
together name vector
1       0       1       0       0       0       H2CO3pKa2 {'HCO3'}
2       0       1       0       0       0       H2CO3pBa2 {'H2CO3'}
0       1       1       0       0       0       logKCUCO3 {'CuCO3'}
0       1       2       0       0       0       logKCUCO32 {'CuCO32'}
1       1       1       0       0       0       logKCUHCO3 {'CuHCO3'}
0       1       0       1       0       0       logKCUCl   {'CuCl'}
0       1       0       0       1       0       logKCuL    {'CuL'}
1       0       0       0       1       0       H2LpKa2   {'HL'}
2       0       0       0       1       0       H2LpBa2   {'H2L'}
0       1       0       0       0       1       logKCUSO4 {'CuSO4'}
0       1       0       0       2       0       logBCuL2  {'CuL2'}
1       1       0       0       1       0       logKCuHL  {'CuHL'}
];

n=size(Tableau,2);
ASOLUTION=cell2mat(Tableau(:,1:n-2));
KSOLUTION=cell2mat(Tableau(:,n-1));
SOLUTIONNAMES=strvcat(Tableau(:,n));

end

% ----- for fixed pH -----

```

```

function
[Ksolution,Asolution]=get_equilib_fixed_pH(KSOLUTION,ASOLUTION,pH)

    [N,M]=size(ASOLUTION);
    Ksolution=KSOLUTION-ASOLUTION(:,1)*pH;
    Asolution=[ASOLUTION(:,2:M)];

end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

function [X,F,J,C] = nl_massbalancerrnosolid_NR(X)

global Asolution Ksolution T

[Nc,Nx]=size(Asolution); %Xsolution=X(1:Nx);
criteria=1e-16;

for i=1:1000

logC=(Ksolution)+Asolution*log10(X); C=10.^(logC); % calc species
R=Asolution'*C-T;

% Evaluate the Jacobian
    z=zeros(Nx,Nx);
for j=1:Nx;
    for k=1:Nx;
        for i=1:Nc;
z(j,k)=z(j,k)+Asolution(i,j)*Asolution(i,k)*C(i)/X(k); end
        end
end

J = z;

deltaX=z\(-1*R);
one_over_del=max([1, -1*deltaX'./(0.5*X')]);
del=1/one_over_del; X=X+del*deltaX;
tst=sum(abs(R));
if tst<=criteria; break; end

end

F=[R];

end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

function [CuT,F]=getdata

```

```

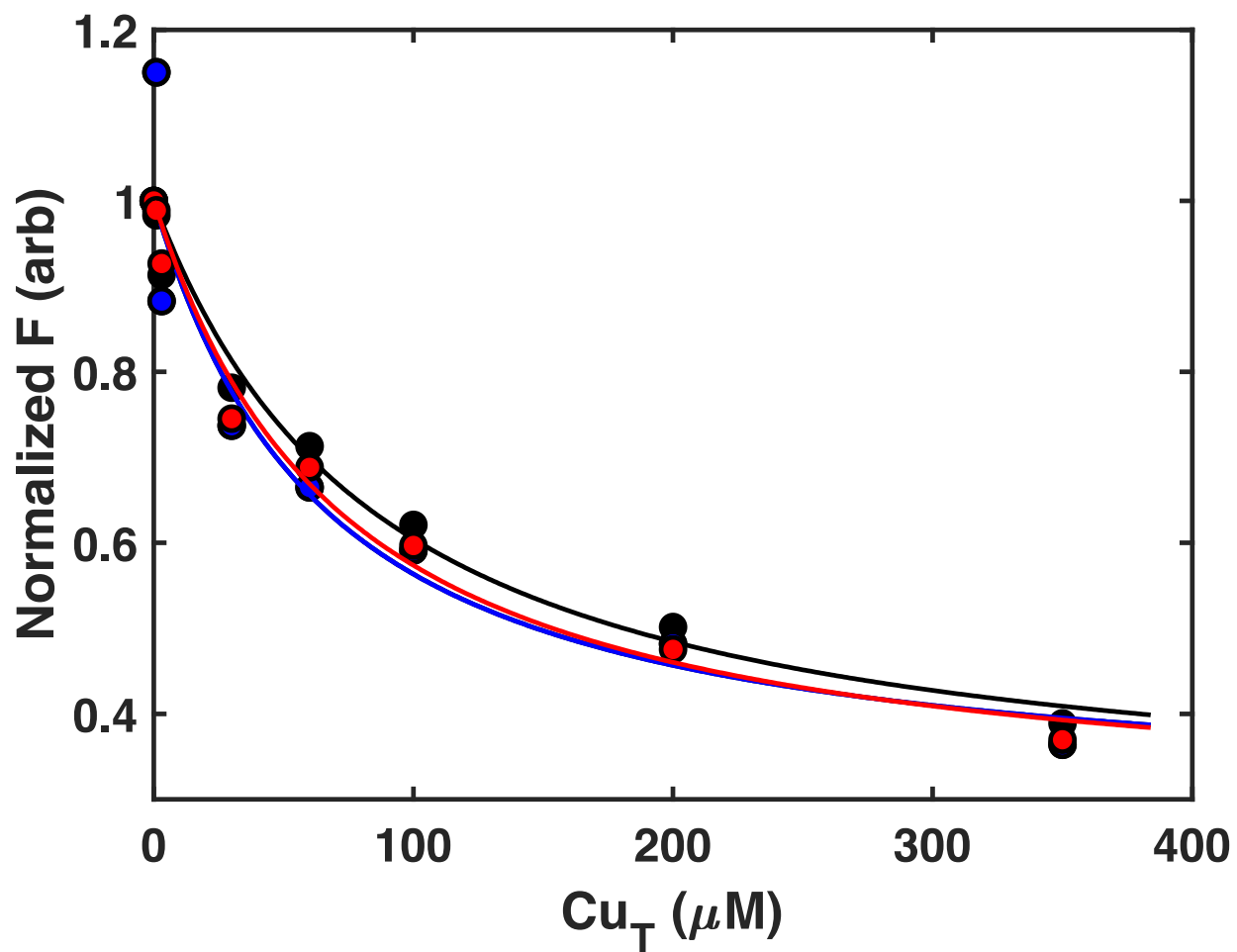
#####
data=[...
% CuT (uM) and F
1.00E-16    1
1    0.988983592
3    0.926690888
%10 0.788341761
30   0.745248443
60   0.688347577
100  0.596926301
200  0.475403332
350  0.369698027
];
#####

CuT=data(:,1)*1e-6; F=data(:,2);
CuT=CuT'; F=F';

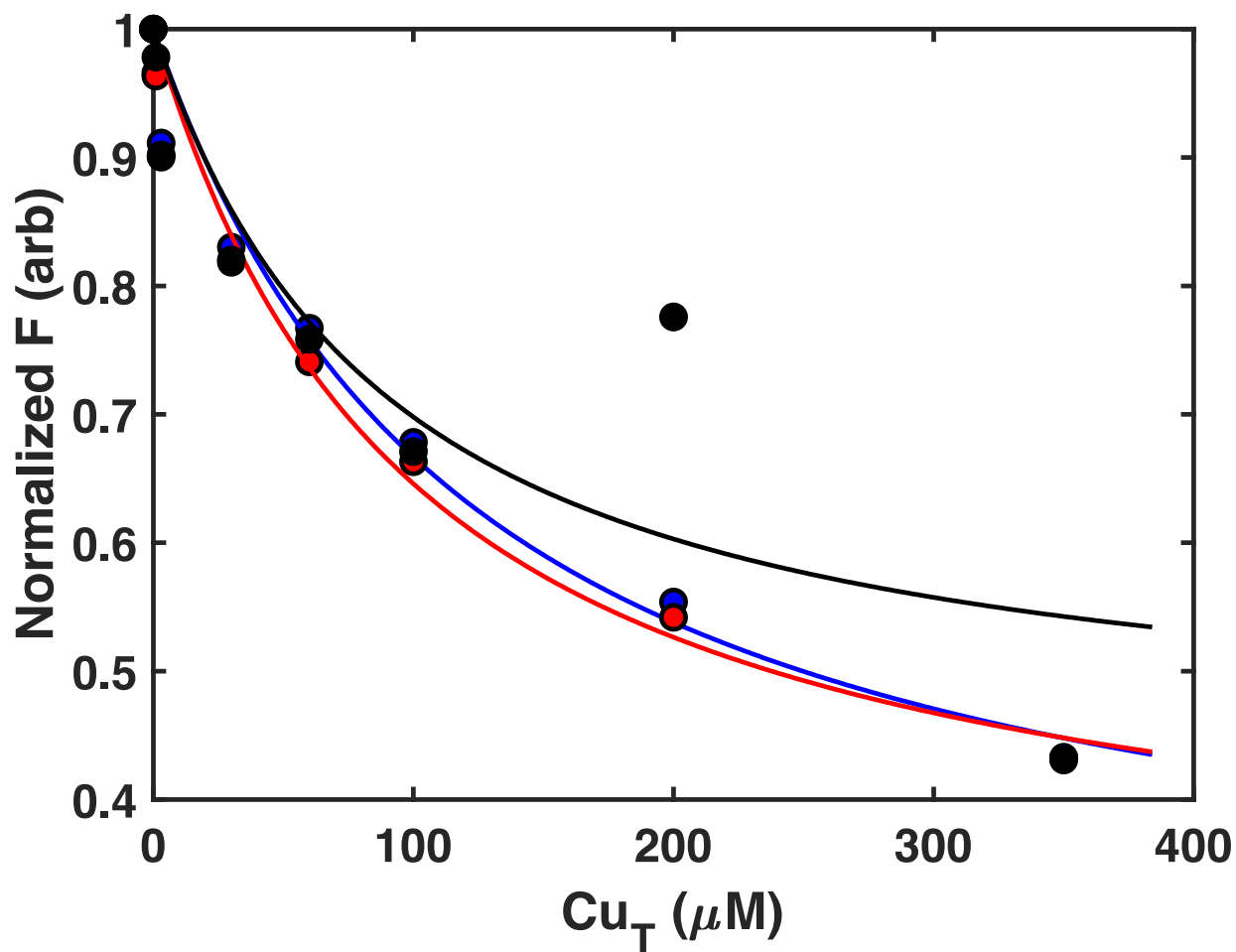
end

```

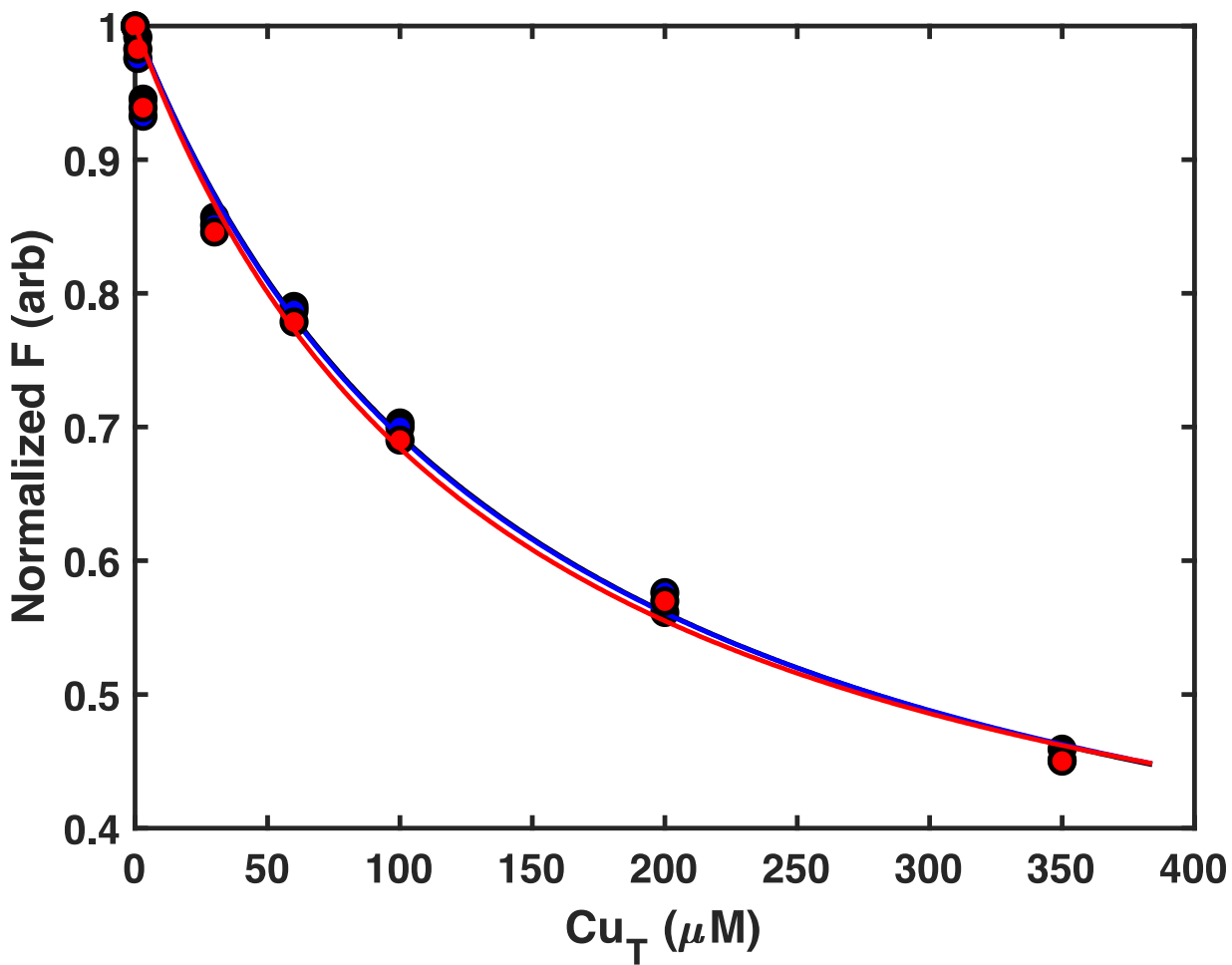
A5. Matlab figures



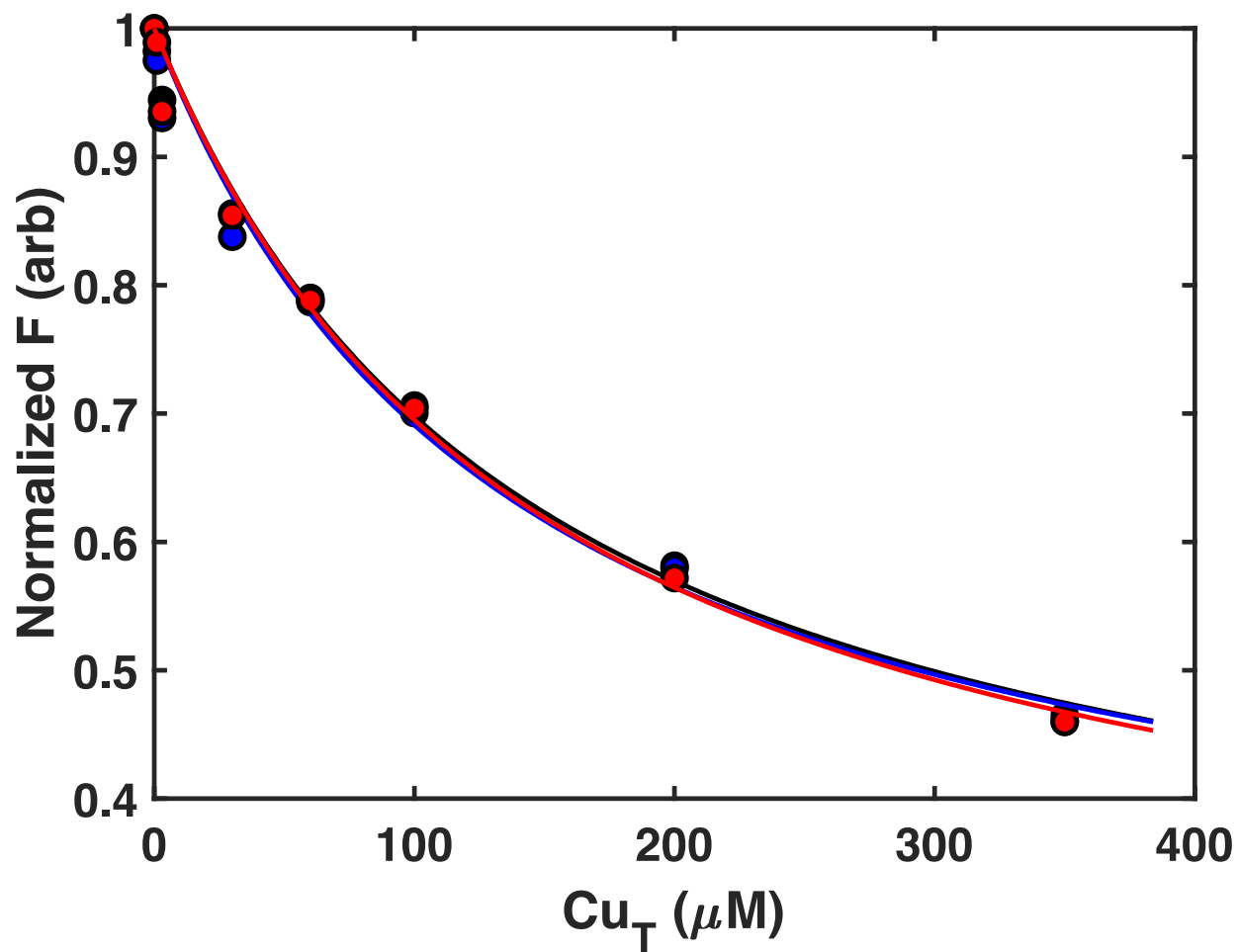
A1. A decrease in the intensity of the fluorescence quenching versus the total concentrations of copper in MQ water.



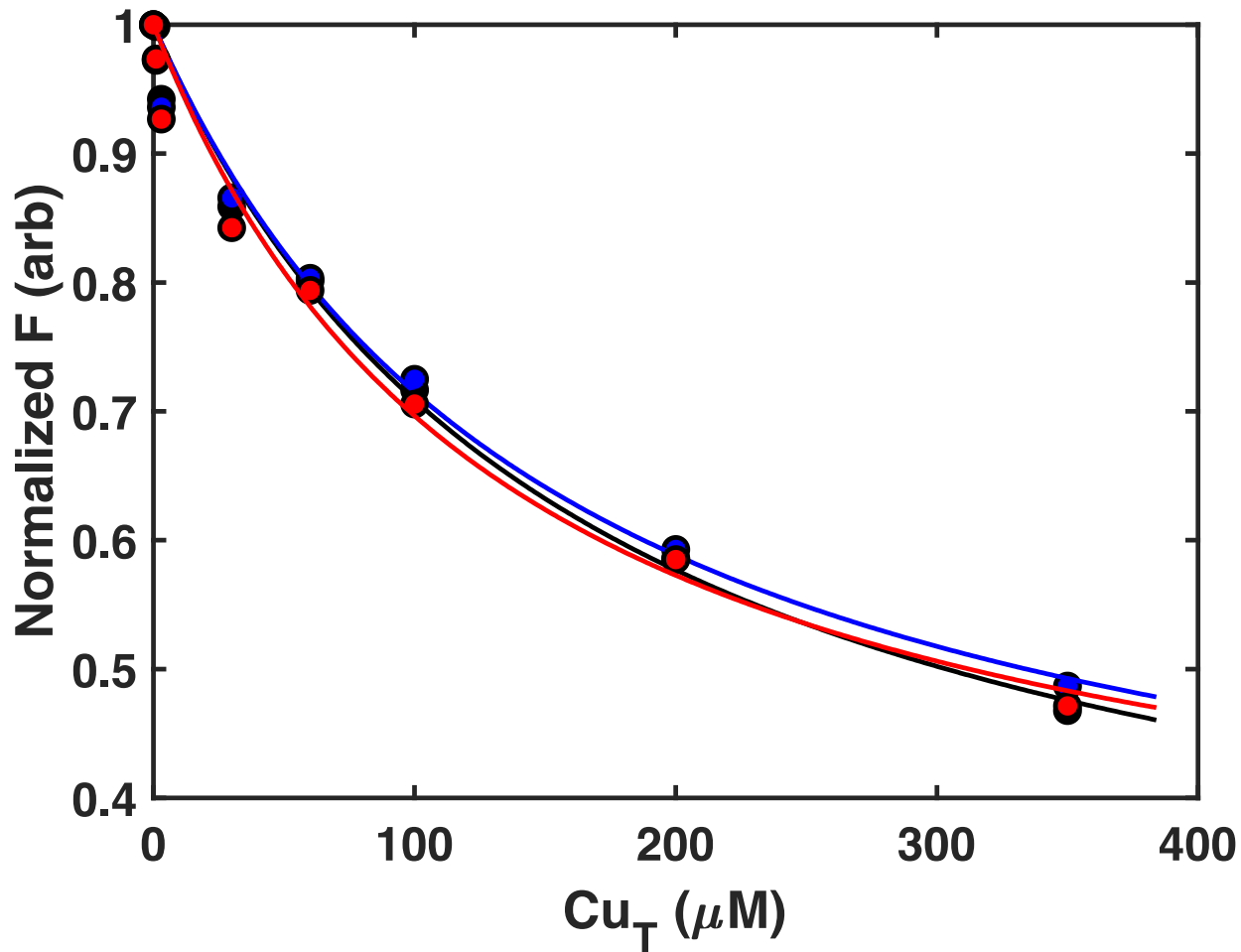
A2. A decrease in the intensity of the fluorescence quenching versus the total concentrations of copper in a solution with (0.01) of Na₂SO₄ salt.



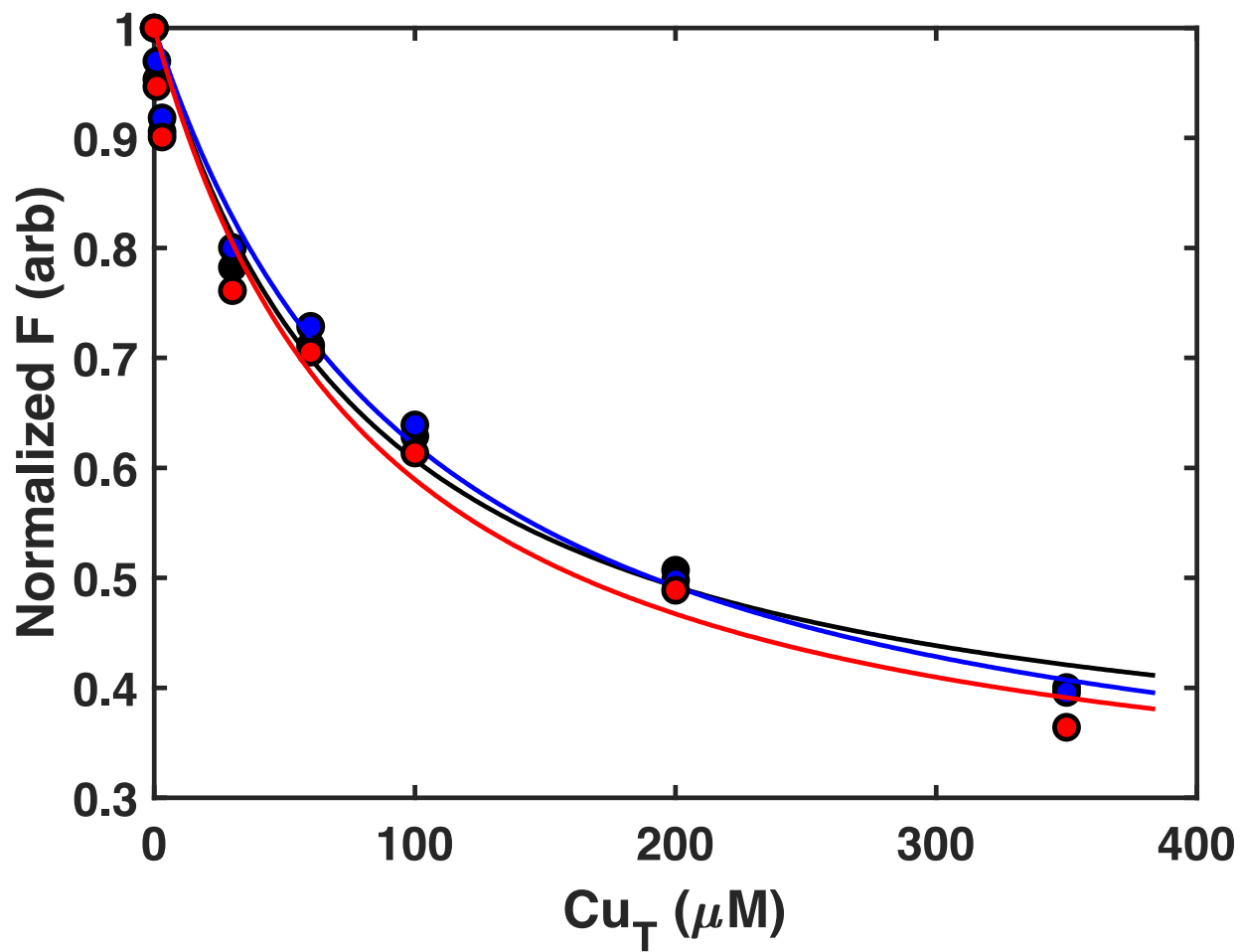
A3. A decrease in the intensity of the fluorescence quenching versus the total concentrations of copper in a solution with (0.05) of Na₂SO₄ salt.



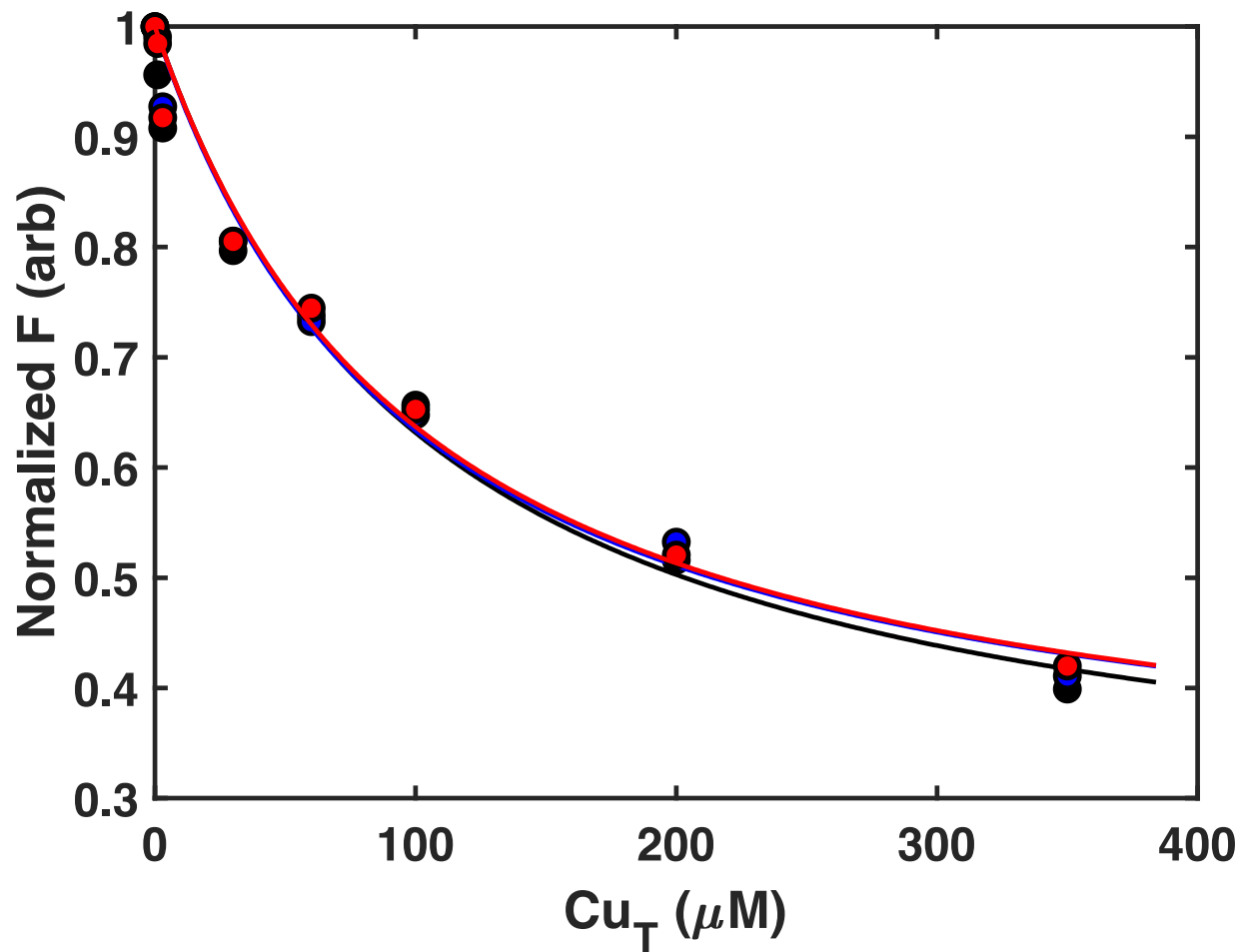
A4. A decrease in the intensity of the fluorescence quenching versus the total concentrations of copper in a solution with (0.25) of Na₂SO₄ salt.



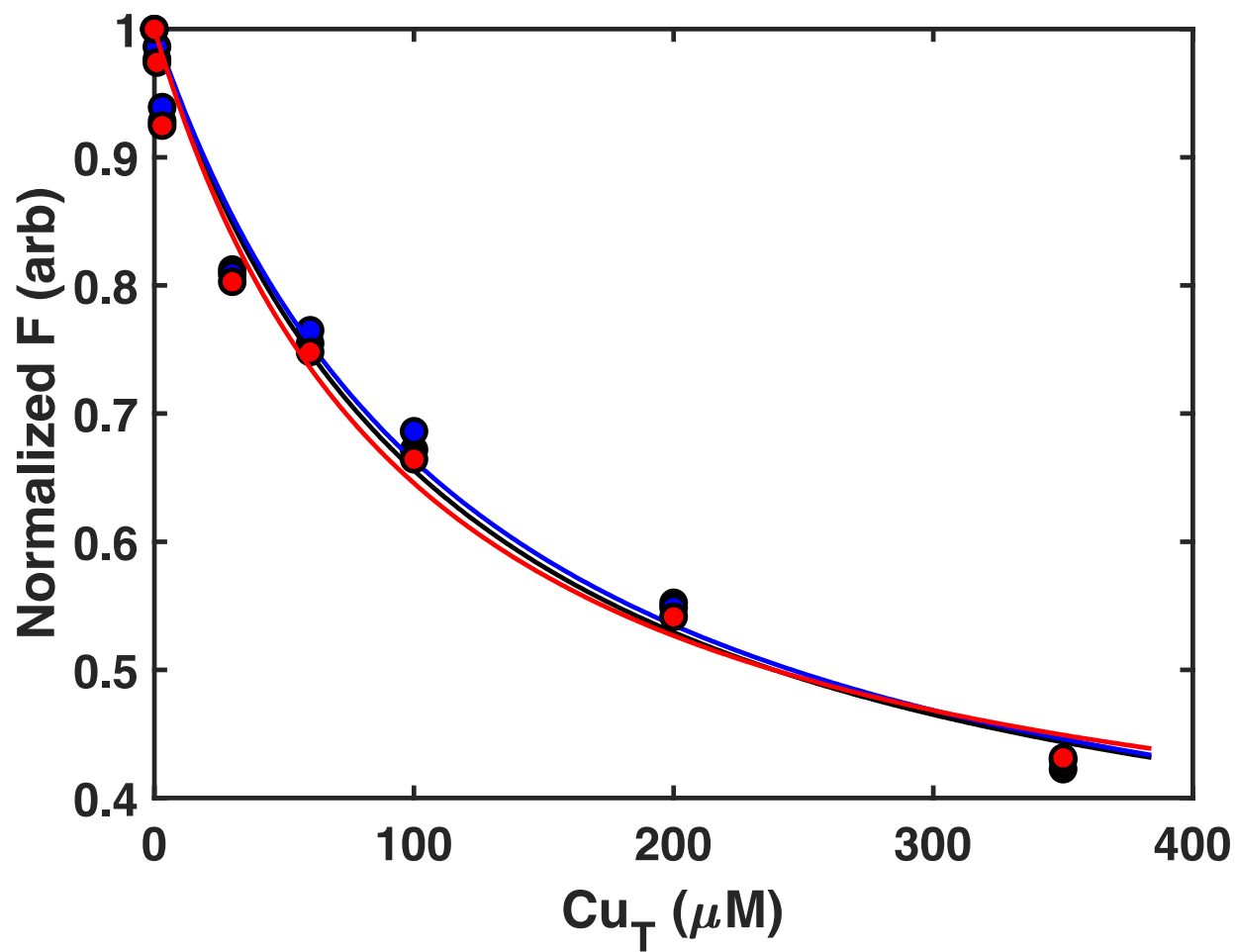
A5. A decrease in the intensity of the fluorescence quenching versus the total concentrations of copper in a solution with (0.4) of Na₂SO₄ salt.



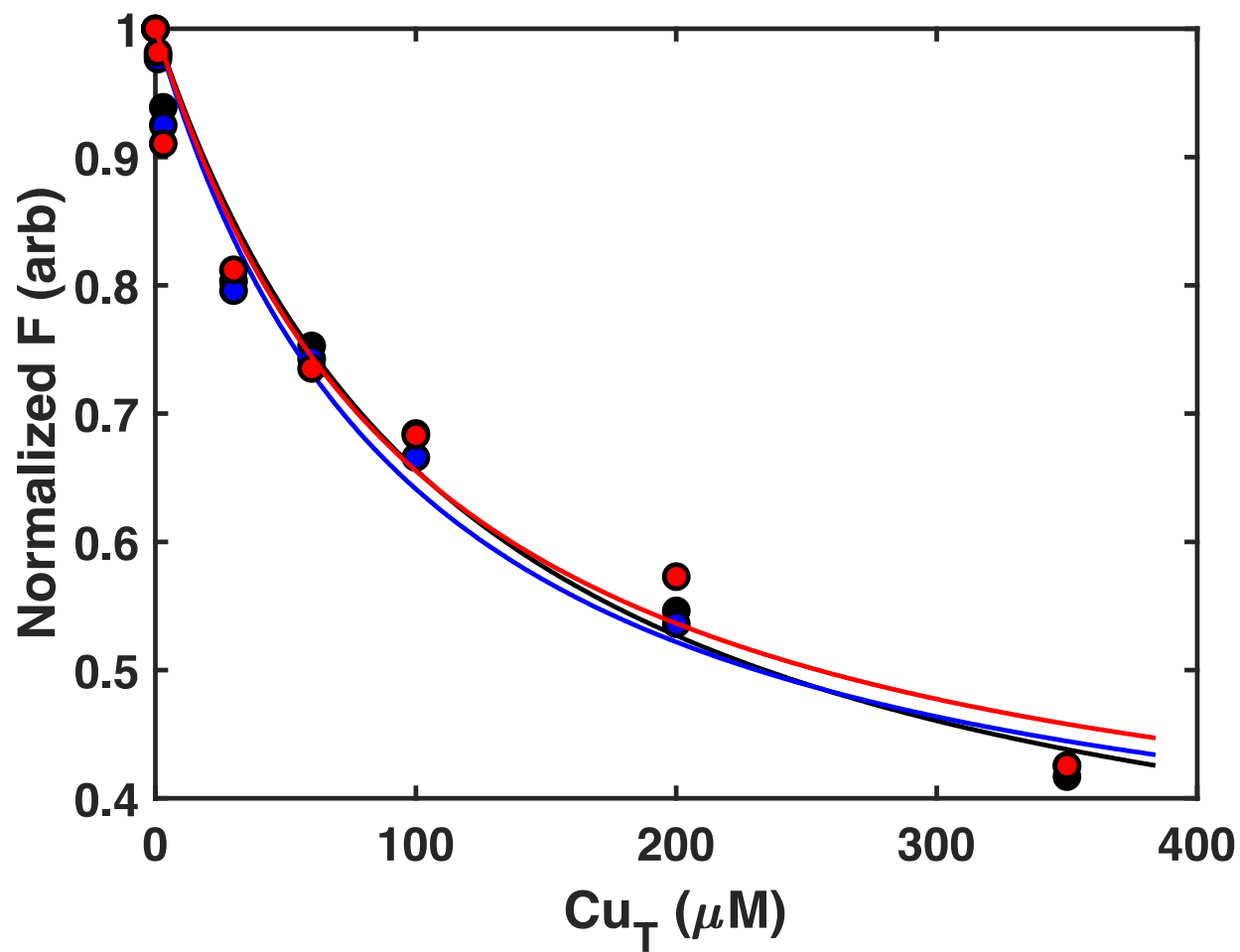
A6. A decrease in the intensity of the fluorescence quenching versus the total concentrations of copper in a solution with (10%) of artificial seawater.



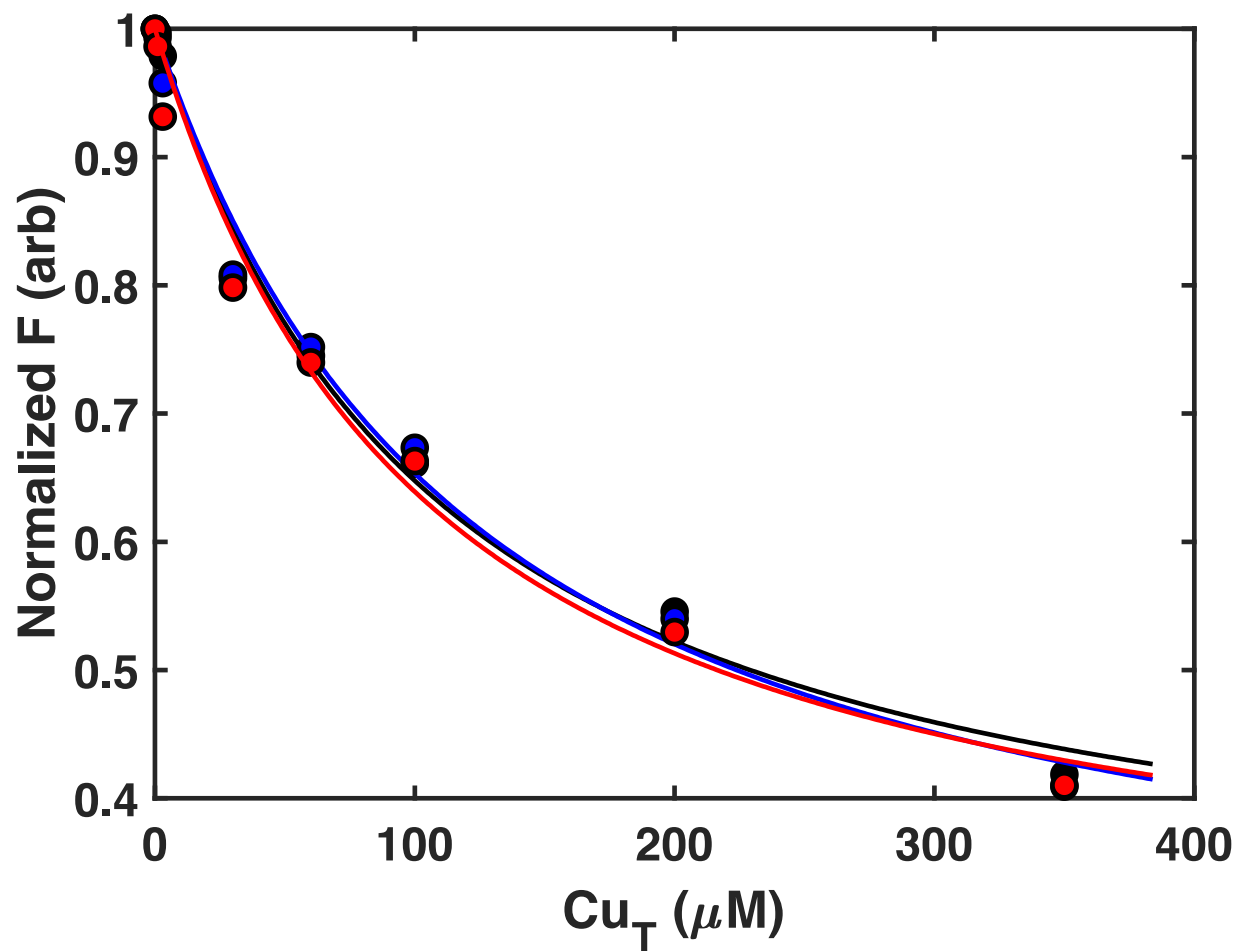
A7. A decrease in the intensity of the fluorescence quenching versus the total concentrations of copper in a solution with (25%) of artificial seawater.



A8. A decrease in the intensity of the fluorescence quenching versus the total concentrations of copper in a solution with (50%) of artificial seawater.



A9. A decrease in the intensity of the fluorescence quenching versus the total concentrations of copper in a solution with (75%) of artificial seawater.



A10. A decrease in the intensity of the fluorescence quenching versus the total concentrations of copper in a solution with (100%) of artificial seawater.

References

- Annex 10: Guidance on transformation/dissolution of metals and metal compounds.* (2007).
Chadwick, D. B., Rivera-Duarte, I., Rosen, G., Wang, P.-F., Santore, R. C., Ryan, A. C., ...
Choi, W. (2008). *Demonstration of an Integrated Compliance Model for Predicting Copper Fate and Effects in DoD Harbors.*
- Coble, P. G. (1996). Characterization of Marine and Terrestrial DOM in Seawater Using Excitation-Emission Matrix Spectroscopy. *Marine Chemistry*, 51(4), 325–346.
- da Silva, J. C. G. ., Machado, A. A. S. C., Oliveira, C. J. S., & Pinto, M. S. S. D. S. (1998). Fluorescence quenching of anthropogenic fulvic acids by Cu(II), Fe(III) and UO₂+2, 45, 1155–1165.
- Day, J. J., Hall, C., Kemp, W., & Janez-Arancibia, A. (1989). *Estuarine Ecology.*
- Di Toro, D. M., Allen, H. E., Bergman, H. L., Meyer, J. S., Paquin, P. R., & Santore, R. C. (2001). Biotic Ligand Model of the Acute Toxicity of Metals. 1. Technical basis. *Environmental Toxicology and Chemistry*, 20(10), 96–2383.
- Engel, D., & Sunda, W. (1979). Toxicity of Cupric Ion to Eggs of the Spot *Leiostomus Xanthurus* and the Atlantic Silverside *Menidia menidia*. *Marine Biology*, 50(2), 121–126.
- Glasby, G., & Schulz, H. (1999). pH Diagrams for Mn, Fe, Co, Ni, Cu and As under seawater conditions: application of two new types of Eh, pH diagrams to the study of specific problems in marine geochemistry. *Aquatic Geochemistry*, 5(3), 227–248.
- González-Dávila, M., Santana-Casiano, J. M., González, A. G., Pérez, N., & Millero, F. J. (2009). Oxidation of copper(I) in seawater at nanomolar levels. *Marine Chemistry*, 115(1–2), 118–124. Retrieved from

https://sfx.scholarsportal.info/laurier?frbrVersion=5&ctx_ver=Z39.88-2004&ctx_enc=info:ofi/enc:UTF-8&ctx_tim=2018-05-22T17%3A24%3A35IST&url_ver=Z39.88-2004&url_ctx_fmt=info:ofi/fmt:kev:mtx:ctx&rfr_id=info:sid/primo.exlibrisgroup.com:primo3-Article-sciverses

Grosell, M., & Wood, C. M. (2002). Copper Uptake Across Rainbow Trout Gills. *Journal of Experimental Biology*, 205(8), 1179–1188.

Harris, D. C. (2010). *Quantitative Chemical Analysis* (8th ed.).

Hooijschuur, J. H. (2018). Fluorescence Spectrometry. Retrieved from

<http://www.chromedia.org/chromedia?waxtrapp=mkqjtbEsHiemBpdmBIIecCArB&subNav=cczbdbEsHiemBpdmBIIecCArBP>

Howard, A. G. (1998). *Aquatic Environmental Chemistry*.

Kogut, M. B., & Voelker, B. M. (2001). Strong Copper-Binding Behavior of Terrestrial Humic Substances in Seawater. *Environmental Science and Technology*, 35, 1149–1156.

Mart, L., Bruland, K. W., & Coale, K. H. (1985). Analysis of Seawater for Dissolved Cadmium, Copper and Lead: An Intercomparison of Voltammetric and Atomic Absorption Methods. *Marine Chemistry*, 17(4), 285–300.

Mason, R. P. (2013). *Trace Metals in Aquatic Systems*.

Merdy, P., Bonnefoy, A., Martias, C., Garnier, C., & Huclier, S. (2012). Use of Fluorescence Spectroscopy and Voltammetry for the Analysis of Metal-Organic Matter Interactions in the New Caledonia lagoon. *International Journal of Environmental Analytical Chemistry*, 92(7), 868–893.

Millero, F. J. (2001). *Physical Chemistry of Natural Waters*.

- Nadella, S. R., Fitzpatrick, J. L., Franklin, N., Bucking, C., Smith, S., & Wood, C. M. (2009). Toxicity of dissolved Cu, Zn, Ni and Cd to developing embryos of the blue mussel (*Mytilus trossulus*) and the protective effect of dissolved organic carbon. *Comparative Biochemistry and Physiology*, 149(3), 340–348.
- NIST. (2010). NIST Critically Selected Stability Constants of Metal Complexes. National Institute of Standards and Technology. Retrieved from <http://www.nist.gov/srd/nist46.cfm>
- OECD. (2001). *Environment Health and Safety Publications* (Testing and Assessment No. 29 Environment Directorate).
- Paquin, P. R., Gorsuch, J. W., Apte, S., Batley, G. E., Bowles, K. C., Campbell, P. G. C., ... Wu, K. B. (2002). The Biotic Ligand Model: A Historical Overview. *Comparative Biochemistry and Physiology*, 133(1), 3–35.
- Ryan, D. K., & Weber, J. H. (1982). Fluorescence Quenching Titration for Determination of Complexing Capacities and Stability Constant of Fulvic Acid. *Analytical Chemistry*, 54(6), 986–990.
- Shirlie, S. (2018). Aquarium Water pH Maintenance.
- Smith, D. S., Arnold, W. R., Cotsifas, J. S., Ogle, S. R., & DePalma, S. G. S. (2010). A COMPARISON OF THE COPPER SENSITIVITY OF SIX INVERTEBRATE SPECIES IN AMBIENT SALT WATER OF VARYING DISSOLVED ORGANIC MATTER CONCENTRATIONS. *Environmental Toxicology and Chemistry*, 29(2), 311–319.
- Smith, D. S., Cooper, C. A., & Wood, C. M. (2017). Measuring Biotic Ligand Model (BLM) Parameters in Vitro: Copper and Silver Binding to Rainbow Trout Gill Cells as Cultured Epithelia or in Suspension. *Environmental Science and Technology*, 51(3), 1733–1741. <https://doi.org/10.1021/acs.est.6b04823>

- Smith, D. S., & Kramer, J. R. (1998). Multi-site aluminum speciation with natural organic matter using multiresponse fluorescence data, *363*, 21–29.
- Smith, D. S., & Kramer, J. R. (2000). Multisite Metal Binding to Fulvic Acid Determined Using MultiResponse Fluorescence. *Analytica Chimica Acta*, *416*(2), 211–220.
- Smith, S., Arnold, R., James, M., Dixon, G., & DePalma, S. (2011). Effects of dissolved organic matter and reduced sulphur on copper bioavailability in coastal marine environments. *Ecotoxicology and Environmental Safety*, (3), 230–237.
- Wang, X., Hua, L., & Ma, Y. (2012). A biotic ligand model predicting acute copper toxicity for barley (*hordeum vulgare*): Influence of calcium, magnesium, sodium, potassium and pH. *Chemosphere*, *89*(1), 89–95.
- Whitfield, A. K. (1999). Ichthyofaunal Assemblages in an Estuarine a South African Case Study. *Reviews in Fish Biology and Fisheries*, *9*(2), 151–186.