GEOCHEMICAL SPECIATION MODELING OF TETRACYCLINE SORPTION AND BIOAVAILABILITY IN THE ENVIRONMENT

By

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ABSTRACT

GEOCHEMICAL SPECIATION MODELING OF TETRACYCLINE SORPTION AND BIOAVAILABILITY IN THE ENVIRONMENT

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The broad-spectrum antibiotic tetracycline is used extensively for human and animal health, but as usual, causes unintended environmental consequences. Since large fractions of tetracycline are not metabolized in animals, massive amounts of tetracycline are excreted with animal manures and pollute soil, surface water, and groundwater. Among other risks, tetracycline induces antibiotic resistance in environmental bacteria. In order to understand and quantify this risk, the objective of this study was to model bacterial uptake of tetracycline in complex media. The speciation of tetracycline is complicated by several ionic species that form complexes with aqueous cations and also with mineral surfaces, so computational tools are needed. This study used many experimental data sets to create thermodynamic parameters for use in the chemical speciation model Phreeqc. Experimental work has shown that the neutral species of tetracycline dominates the uptake by bacteria, so a new method for modeling the distribution of organic chemicals using Phreeqc was employed to successfully model bacterial uptake in complex solutions of Ca^{2+}, Mg^{2+}, and five organic acids. Since clay minerals are important sorbents, cation exchange parameters were developed for tetracycline and its K- and Ca-complexes. While experimental data for K- or Ca-systems could be successfully fit separately, no parameter set could be found that fit both together over a range of four ionic strengths. This calls into question the literature values for Ca^{2+}/K^{+} exchange itself, which provides a path forward for future research.

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Chapter 1

Modeling the Bioaccumulation of Tetracycline into *E.coli*

1.1 Introduction

In the 20th century, antibiotics were major new discoveries in terms of human and animal medicine. In the world, including the United States, antibiotics protect the lives of millions of humans and animals every year [8]. In 2010, the usage of antimicrobials in animal foods was around 13.5 million kg [9].

Tetracycline is a broad-spectrum antibiotic that was discovered in early of 1950s [10]. The chemical formula of tetracycline consists of four fused cyclic rings with attached functional groups, including dimethylammonium, tricarbonylmethane, and diketone (Figure1.1). In the aqueous phase, these functional groups often become ionized and cause tetracycline to form cation, zwitterion (neutral), or anion species, depending on solution pH (Figure 1.2).

Tetracycline has extensive uses for human and animal health by protecting them from a variety of infectious diseases. Tetracycline usage comprised 42% of the total antimicrobials used in food animal production, $5,602,281$ kg of tetracycline in 2010 [9]. Since large fractions of tetracycline are not metabolized in the animals, massive amounts of tetracycline are excreted with animal manures, either as the original compound or as its bioactive metabolites

[11, 12]. Thus, tetracycline and its derivatives are transferred to soil [13, 14], surface water [15–18], and groundwater [14, 19, 20].

In the environment, tetracycline speciation in aqueous solutions can be very complex. In the simplest case, tetracycline in pure water already contains four species, depending on pH (Figure 1.2). Typically, aqueous solutions will also contain a variety of metal cations, including Ca^{2+}, Mg^{2+}, and K^{+}. These cations may form complexes with tetracycline, thus reducing the activity of uncomplexed tetracycline and forming additional species that must be considered (Figure 1.3). The activities of metal ions may be altered by the presence of organic ligands that can complex the metals, or by sorption of the metals to solid phases such as clay minerals or organic matter. Such competitive complexation or sorption of metals will generally increase the activity of aqueous uncomplexed tetracycline. Finally, tetracycline itself may sorb to a variety of soil minerals (e.g., smectite clays, goethite, or magnetite) and to soil organic matter, thus decreasing tetracycline activity in solution.

Among the different species of tetracycline present in aqueous solution, including cationic species of tetracycline and neutral or zwitterionic species, Zhang et al. [4] hypothesized that the neutral species was the dominant form taken up by bacterial (*Escherichia coli*) cells.The neutral, zwitterionic species of tetracycline would thus acquire great importance in attempts to understand the development of antibiotic resistance in bacteria. It would also mean that geochemical components like pH and cations have an enormous influence on the induction of bacterial resistance [4].

Though tetracycline levels discovered in many environmental media are lower than the levels required to show minimum inhibitory concentration (MIC) for medicinal interests, observed environmental tetracycline levels probably do discriminate among bacteria by stressing populations [19, 21–24]. The result is that bacteria containing the genes for antibiotic

resistance will be more likely to survive, which will enhance the antibiotic resistance of bacterial inhabitants in our environment [19, 21–24]. This is a serious global problem. Early in 2014, the World Health Organization published a cautionary report that the people are in danger of finding themselves in a post-antibiotic era [25]. Also, the report has said that doctors are unable to fight infections like drug-resistant gonorrhea, and we need modern treatments to substitute for the drugs which bacterial resistance has made ineffective [25]. The usages of antibiotics are increasing but still imperfect to hold up with the resistance of bacteria [26].

The resistance of bacteria is complicated and involves several aspects which can cause many human and animal diseases [25]. In the United States itself, around 2 million diseases and 23 thousand deaths happened due to the bacterial resistance [8]. If we lose the benefits of antibiotics, we will be incapable to treat the infections of bacteria [8]. Therefore, in 2014, the U.S. government has created a national strategy and an action plan to face and treat this serious problem by several different steps [8].

In order to understand this complexity of tetracycline speciation in environmental waters, computational tools are needed to iteratively and quantitatively estimate the distribution of tetracycline among its many possible species. Phreeqc is a tool that was developed over 35 years and continues to be improved [27] for thermodynamic modeling of aqueous environmental systems. Phreeqc is a public-domain tool that has already been integrated with saturated- and unsaturated-flow water transport modules [28], so it provides a framework for future reactive-transport modeling.

Thermodynamic modeling, as in Phreeqc, is very useful because even the most sophisticated analytical procedures such as liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS), [29] simply measure the total tetracycline concentration rather than

the component tetracycline species. Thermodynamic approaches allow a more powerful interpretation of these analytical LC-MS-MS data by estimating the distribution of tetracycline among its possible species. Therefore, Phreeqc modeling is a method for extracting the most value from data in order to better understand the complexation, sorption, and bioavailability of antibiotics in the environment.

The objective of the current study was to develop a quantitative model for tetracycline speciation in complex systems. A main goal was to be able to predict bacterial uptake of tetracycline as a function of realistic geochemical variables, because bacterial uptake is correlated very strongly with the induction of antibiotic resistance [4]. While many of the relevant chemical equilibria have already been described, a new method was applied and fit to the available data in order to describe bacterial uptake of tetracycline. This appears to be the first implementation of a method to describe linear distribution reactions in Phreeqc, and should be very broadly applicable to modeling the fate of organic solutes in complex geochemical systems.

1.2 Methods

For developing the equations necessary to model the chemical speciation of tetracycline, the recent datasets of Zhang et al. [4, 5] are robust in that they recorded tetracycline, metal (Ca^{2+} and Mg^{2+}), and organic ligand (acetate, oxalate, succinate, malonate, and citrate) concentrations as well as pH and measurements of tetracycline uptake by *E.coli* bacteria. The results of Zhang et al. [4] gave rise to the hypothesis that a single species of tetracycline

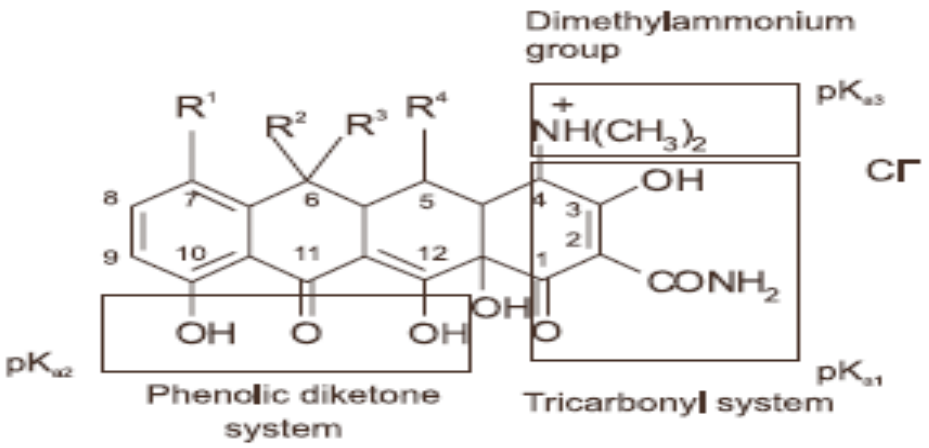

Figure 1.1 Tetracycline chemical structure [1]. Where R^1= H, R^2= CH_3, R^3= OH, and R^4= H [2].

(the neutral, zwitterionic species H_3Tec^0) dominates the bacteria uptake. That is, the significant positive correlation (Figure 1.4) between intracellular tetracycline concentration and H_3Tec^0 indicated that zwitterionic tetracycline was clearly the most favorable species for uptake by the *E.coli* according to bioreporter technique [4, 5]. The uptake of all other tetracycline species seemed to be either inconsequential or even negatively correlated with bacterial uptake.

The present study adjusted the reported [4, 5] initial concentrations of tetracycline for the reported bacterial uptake of tetracycline to yield uptake-adjusted estimates for the final equilibrium aqueous concentrations of tetracycline. For each final, equilibrium aqueous concentration of tetracycline, Phreeqc was then used to estimate the fractional distribution of tetracycline species in the LB medium. All chemical equilibria used for Phreeqc in the present study are listed in Table 1.1.

The relationship of bacterial uptake to aqueous H_3Tec^0 activity found by Zhang et al. [4] was, at a first approximation, linear (Figure 1.4). Therefore, the present study tentatively modeled bacterial uptake using the simple expression:

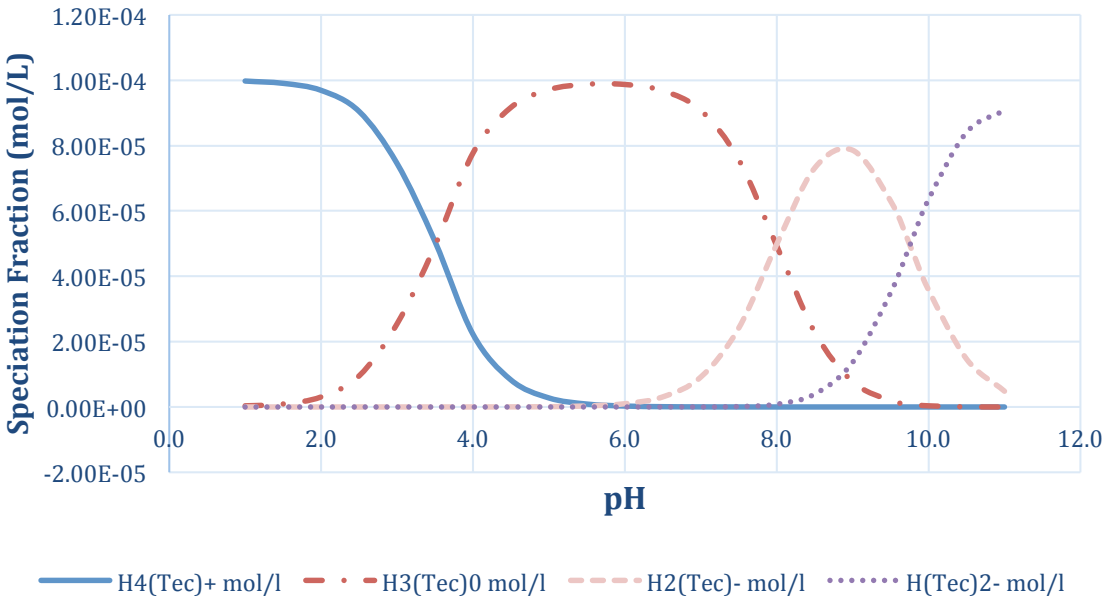

Figure 1.2 Tetracycline species distribution in pure water as a function of solution pH calculated using the Phreeqc parameters from Table 1.1.

$$(\text{BacteriaTetracycline}) \cong K_d(H_3Tec^0_{aq}) \tag{1.1}$$

so that

$$K_d \cong \frac{(\text{BacteriaTetracycline})}{(H_3TeC^0_{aq})} \tag{1.2}$$

where K_d is the distribution coefficient. This simple approach seems appropriate because Equations 1.1 and 1.2 were developed for the distribution of neutral organic compounds into soil organic matter. In the present case, we used the neutral species of tetracycline distribution into bacteria cells, which are reasonable analogues of soil organic matter.

1.2.1 Modeling Tetracycline Uptake by Bacteria in Phreeqc

Again, bacterial uptake of tetracycline limited to be controlled by just one tetracycline species [4]. The functional relation between uptake and zwiterionic tetracycline seemed to

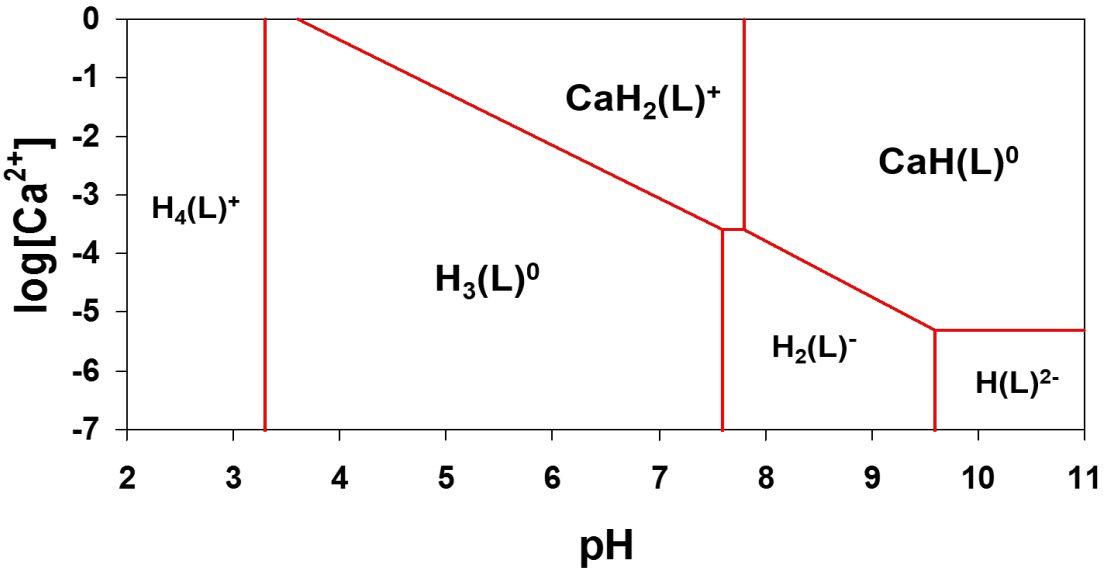

Figure 1.3 Dominant species of tetracycline as a function of pH and Ca^{2+} concentration in aqueous solution [3]. L=tetracycline.

be approximately linear (Figure 1.4), so that it could be approximated with Eq. 1.1 and Eq. 1.2. Therefore, the study adopted an approach for modeling linear adsorption in Phreeqc that has been presented by Appelo and Postma [6], but has apparently never been reported in subsequent literature. This approach could be used to describe any linear distribution reaction, and the present study applies it to describe the uptake of tetracycline by bacteria. If a tetracycline molecule in a bacterial cell is designated "BacteriaTetracycline", then the distribution of tetracycline into bacteria can be expresses as the following process:

$$H_3Tec^0_{aq} + \text{Bacteria} = \text{BacteriaTetracycline} \tag{1.3}$$

Then,

$$K_{eq} = \frac{(\text{BacteriaTetracycline})}{(H_3Tec^0)(\text{Bacteria})} \tag{1.4}$$

But for distribution,

$$K_d = \frac{(\text{BacteriaTetracycline})}{(H_3Tec^0_{aq})} \tag{1.5}$$

That is, $K_{eq} = K_d$ as long as (Bacteria) = 1.00 in Eq. 1.4.

In the "Sorption" model of Phreeqc, the present study created a "Surface" (a phase that can sorb molecules) called "Bacteria". This phase is assigned a certain number (call it "TotalSites") of possible sorption sites. Following the standard conventions for defining the activities of sites in solid solutions [30], the activity of a certain type of site i like this in the solid phase is approximated as its fractional occupancy [30].

$$\text{Activity of site i} \cong \frac{\text{\# sites of type i}}{\text{TotalSites}} \tag{1.6}$$

If the present study considers only the sorption of tetracycline, then the only two types of sites in bacteria will be the occupied "BacteriaTetracycline" sites and the unoccupied "Bacteria" sites, so

$$\text{TotalSites} = \text{\# BacteriaTetracycline} + \text{\# Bacteria} \tag{1.7}$$

Thus, the activity or fraction of the site-type "Bacteria" is:

$$(\text{Bacteria}) = \frac{\text{\# Bacteria}}{\text{\# BacteriaTetracycline} + \text{\# Bacteria}} \tag{1.8}$$

However, if # BacteriaTetracycline $\lll$ # Bacteria, then

$$(\text{\# Bacteria}) \cong \text{\# BacteriaTetracycline} + \text{\# Bacteria} \tag{1.9}$$

And this means that:

$$(\text{Bacteria}) = \frac{\#\text{ Bacteria}}{\#\text{ BacteriaTetracycline} + \#\text{ Bacteria}} \cong \frac{\#\text{ Bacteria}}{\#\text{ Bacteria}} = 1.00 \qquad (1.10)$$

If this is true, then $K_{eq} = K_d$ in Equations 1.4 and 1.5, and the Phreeqc speciation model possesses a chemical equilibrium model for distribution. Thus, the distribution of tetracycline into bacterial cells is treated as if the bacteria contained a large number of individual sites, of which just a few are occupied by tetracycline. Note that if the aqueous concentration of tetracycline is about 100 $\mu g/L$, then there are only $2.3e^{-7}$ mole/L of tetracycline. In the real systems studied by Zhang et al. [4, 5], there were about 200 mg (dry weight) bacteria per L of solution (Zhang, personal communication), so if one assumed the bacterial biomass to be half carbon, then there were about 0.0085 moles bacterial carbon per liter of solution. The bacterial cells thus comprised on the order of 40,000 moles of carbon for each mole of tetracycline in the total system. Thus, it is plausible that $\#\ BacteriaTetracycline <<< \#\ Bacteria$. For the present study, the relevant Phreeqc parameter assigned the bacteria to have 10 moles of sites per L solution, which is huge compared to the amount of tetracycline, in order to guarantee that:

$\#\ BacteriaTetracycline <<< \#\ Bacteria$ is always true and $(Bacteria) = 1.0000$.

1.3 Results and Discussion

Figure 1.5 shows the measured bacterial uptake of tetracycline as a function of the uptake-adjusted, Phreeqc-modeled equilibrium H_3Tec^0 activity. The 130 experimentally derived points in Figure 1.5 correspond to initial tetracycline concentrations ranging from 25 to 125

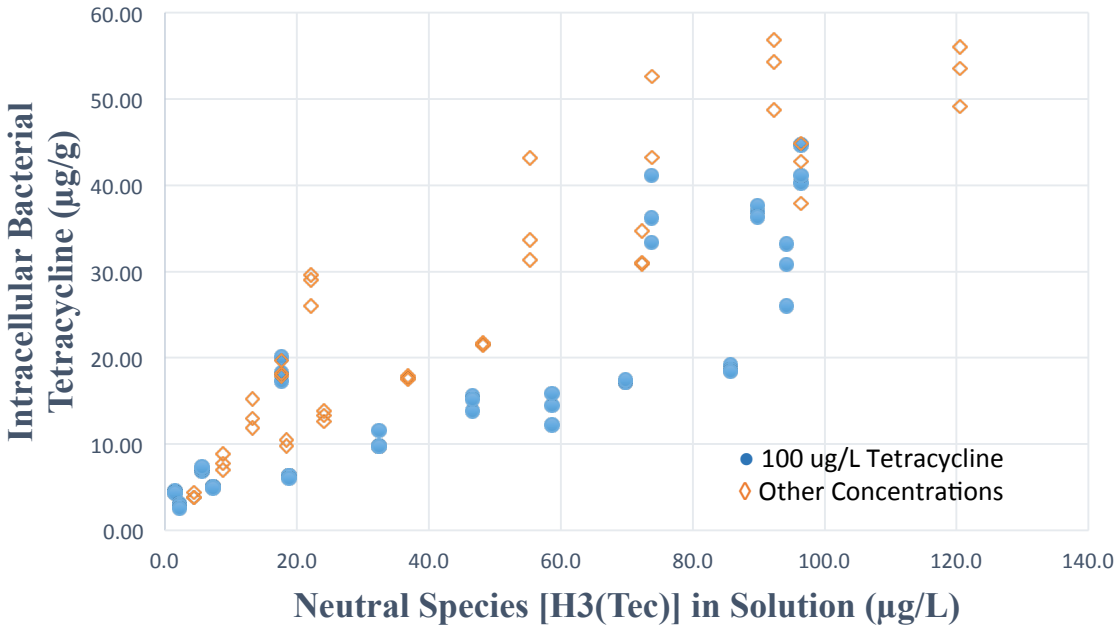

Figure 1.4 Approximate relationship of tetracycline neutral species (zwitterionic species) concentration in solution with the intracellular (*E.coli*) tetracycline concentration. These data cover a wide range of Ca^{2+} or Mg^{2+} concentrations from 0 to 5.0 mmol/L and pH from 6 to 8 [4]. Solution-phase concentrations have not been corrected for bacterial uptake.

$\mu g/kg$ (ppb), pH values from 6 to 8, Ca^{2+} and Mg^{2+} concentrations from 0.1 to 5.3 mmol/L, and concentrations of acetate, oxalate, succinate, malonate, and citrate varying from 0 to 20 mmol/L [14, 29]. The best linear fit to the data had an intercept of 1.9 $\pm$ 3.6 $\mu g/g$ (ppm). Thus, the 95% confidence interval for the intercept was larger than the intercept itself. Since the intercept is not significantly different from zero, the origin of the line at zero. In this case, Eq. 1.1 and Eq. 1.2 applied; a linear regression of the data in Figure 1.5, forced through the origin, yielded a slope and 95% confidence interval of $K_d = 524 \pm 35$ L/kg.

The Phreeqc method used in this study accurately modeled the bacterial uptake of the tetracycline neutral species H_3Tec^0 in solution and bacterial uptake (Figure 1.5). After adjusting the data (Figure 1.4), the data continued to mirror the linear relationship obtained by Zhang et al. (Figure 1.5). The distribution relationship developed for Phreeqc is represented by the line in Figure 1.5. Furthermore, to our knowledge, no other researchers have

used this approach for distribution of organics into bacteria or organic matter using Phreeqc. Therefore, the approach of this study will help interested researchers in the fields of emerging contaminants and remediation of soil and water pollution.

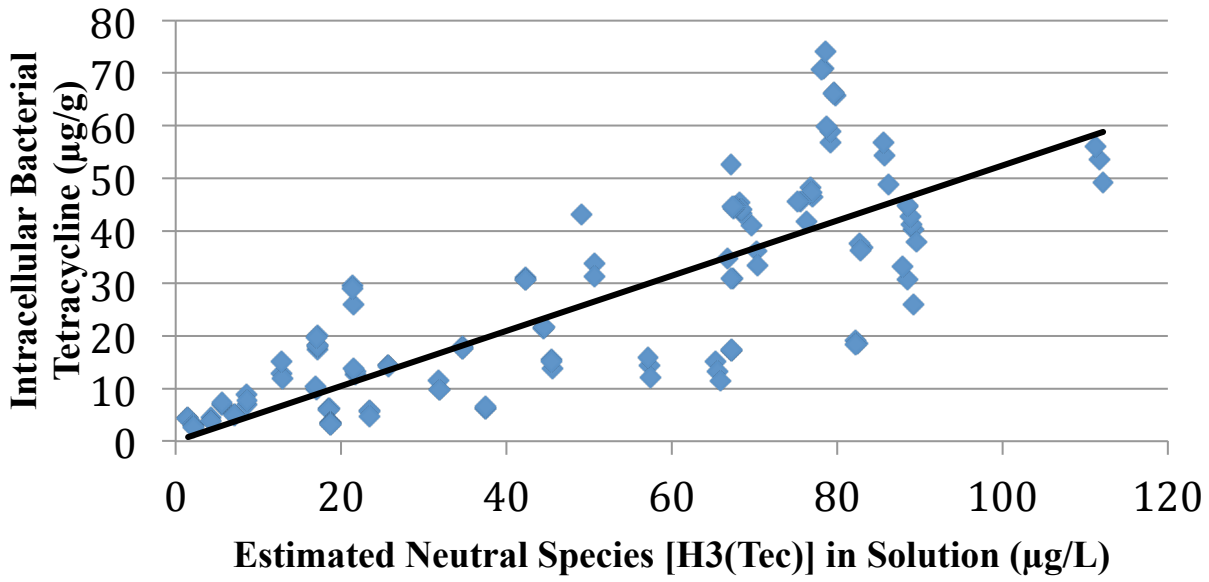

Figure 1.5 Results of modeling the relationship between tetracycline neutral species (zwitterionic species) concentration in solution and intracellular (*E.Coli*) tetracycline concentration using Phreeqc. These data cover a wide range of Ca^{2+} and Mg^{2+} concentrations from 0 to 5.0 mmol/L and pH from 6 to 8 [4], and include solutions containing five different organic acids at pH 7 [5]. Solution-phase concentrations have been corrected for bacterial uptake. The dark line is both the linear regression of the data and the Phreeqc prediction for bacterial uptake of tetracycline in each of the 130 systems.

The key assumption made in the sorption module of Phreeqc to model the distribution of tetracycline into bacteria is that the number of sorption sites is much larger than the number of molecules of tetracycline sorbed. This assumption should be valid because distribution is similar to a process of dissolution [31]. That is, the capacity of the bacterial phase for tetracycline grows even larger as tetracycline becomes sorbed. As discussed above, there were perhaps 40,000 moles of bacterial carbon per mole of tetracycline sorbed, so it seems the assumption should be valid. Distribution is a very important geochemical process, yet the literature seems to contain no previous examples of using Phreeqc to model distribution. This

work shows that this Phreegc model can be successfully applied to hydrophobic distribution.

1.4 Conclusions

A novel method was adapted to model the distribution of the neutral (zwitterionic) species of tetracycline into bacterial cells. The method was applied to a range of pH values $(6-8)$, a range of concentrations of Ca^{2+} and Mg^{2+}, and varying five organic acids that can complex the metals in competition with tetracycline. This study demonstrated that the method of Appelo and Postma [6] can be effectively used to model distribution using Phreeqc. Given that sorption of many hydrophobic organic compounds is dominated by distribution, this functionality in Phreeqc may have widespread application. Furthermore, using Phreeqc we successfully modeled the hypothesis of Zhang et al. [4, 5] that the uptake of tetracycline by *E.coli* can be approximated as a process of distribution of a single tetracycline species, namely the neutral, zwitterionic form.

Table 1.1 Compilation of all chemical equations and equilibrium constants used in the present study. All equations are written as they appear in the Phreeqc database.

Section in Phreeqc Database	Reactions	log_k
SOLUTION_MASTER SPECIES	Tec $H_4(Tec)^+$	—
	$H_4(Tec)^+ = H_4(Tec)^+$	0.000
	$H_4(Tec)^+ = H_3(Tec)^+ H^+$	-3.45^a
	$H_4(Tec)^+ = H_2(Tec)^- + 2H^+$	-11.45^a

Table 1.1 (cont'd)

Section in Phreeqc Database	Reactions	log_k
SOLUTION_SPECIES	$H_4(Tec)^+ = H(Tec)^{2-} + 3H^+$	-21.23^a
	$H_4(Tec)^+ = Tec^{3-} + 4H^+$	-33.64^a
	$Ca^{2+} + H_2(Tec)^- = CaH_2(Tec)^+$	3.4^b
	$Mg^{2+} + H_2(Tec)^- = MgH_2(Tec)^+$	3.9^b
	$\mathbf{Ca^{2+} + H(Tec)^{2-} = CaH(Tec)}$	5.8^b
	$\mathbf{Mg^{2+} + H(Tec)^{2-} = MgH(Tec)}$	4.1^b
	$\mathbf{Ca^{2+} + H_3(Tec) = CaH_3(Tec)^{2+}}$	3.0^d
	$K^+ + H_2(Tec)^- = KH_2(Tec)$	1.04^e
	$\mathbf{K^+ + H_3(Tec) = KH_3(Tec)^+}$	1.04^e
	$Oxalate^{2-} + H^+ = HOxalate^-$	4.27^g
	$HOxalate^- + H^+ = H_2Oxalate$	1.25^c
	$Ca^{2+} + Oxalate^{2-} = Ca(Oxalate)$	3.0^g
	$Mg^{2+} + Oxalate^{2-} = Mg(Oxalate)$	3.43^g
	$Malonate^{2-} + H^+ = HMalonate^-$	5.70^c
	$HMalonate^- + H^+ = H_2Malonate$	2.85^c
	$Ca^{2+} + Malonate^{2-} = Ca(Malonate)$	2.35^c
	$Mg^{2+} + Malonate^{2-} = Mg(Malonate)$	2.85^c
	$Succinate^{2-} + H^+ = HSuccinate$	5.64^c
	$HSuccinate^- + H^+ = H_2Succinate$	4.21^c
	$Ca^{2+} + Succinate^{2-} = Ca(Succinate)$	2.0^c

Table 1.1 (cont'd)

Section in Phreeqc Database	Reactions	log_k
SOLUTION_SPECIES	$Mg^{2+} + Succinate^{2-} = Mg(Succinate)$	2.0^{c}
	$H^{+} + Acetate^{-} = H(Acetate)$	4.757^{f}
	$Mg^{2+} + Acetate^{-} = Mg(Acetate)^{+}$	1.27^{g}
	$Ca^{2+} + Acetate^{-} = Ca(Acetate)^{+}$	1.18^{g}
	$H^{+} + Citrate^{3-} = H(Citrate)^{2-}$	6.396^{f}
	$2H^{+} + Citrate^{3-} = H_2(Citrate)^{-}$	11.157^{f}
	$3H^{+} + Citrate^{3-} = H_3(Citrate)$	14.258^{f}
	$Ca^{2+} + Citrate^{3-} = Ca(Citrate)^{-}$	4.87^{f}
	$Ca^{2+} + Citrate^{3-} + H^{+} = CaH(Citrate)$	9.26^{f}
	$Ca^{2+} + Citrate^{3-} + 2H^{+} = CaH_2(Citrate)^{+}$	12.257^{f}
	$Mg^{2+} + Citrate^{3-} = Mg(Citrate)^{-}$	4.89^{f}
	$Mg^{2+} + Citrate^{3-} + H^{+} = MgH(Citrate)$	8.91^{f}
	$Mg^{2+} + Citrate^{3-} + 2H^{+} = MgH_2(Citrate)^{+}$	12.2^{f}
PHASES	Fix_H^{+} $H^{+} = H^{+}$	0.000
SURFACE_MASTER SPECIES	# Bacteria is to allow distribution of antibiotic into bacterial cells Bacteria Bacteria	—
SURFACE_SPECIES	Bacteria = Bacteria	0

Table 1.1 (cont'd)

Section in Phreeqc Database	Reactions	log_k
	$\mathbf{Bacteria} + \mathbf{H_3(Tec)} = \mathbf{BacteriaH_3(Tec)}$	-1.2805^{d}
EXCHANGE_MASTER SPECIES	$X \quad X^-$	—
EXCHANGE_SPECIES	$X^- \quad X^-$	0.0^{f}
	$\mathbf{H_4(Tec)^+ + X^- = H_4(Tec)X}$	3.8^{d}
	$\mathbf{CaH_2(Tec)^+ + X^- = CaH_2(Tec)X}$	7.0^{d}
	$\mathbf{\#CaH_3(Tec)^{2+} + 2X^- = CaH_3(Tec)X_2}$	$\#4.2^{d}$
	$\mathbf{MgH_2(Tec)^+ + X^- = MgH_2(Tec)X}$	2.0^{d}
	$\mathbf{KH_3(Tec)^+ + X^- = KH_3(Tec)X}$	3.6^{d}
	$Na^+ + X^- = NaX$	0.0^{f}
	$K^+ + X^- = KX$	0.7^{f}
	$Ca^{2+} + 2X^- = CaX_2$	0.8^{f}
	$Mg^{2+} + 2X^- = MgX_2$	0.6^{f}

[a]data from Werner et al. 2006 were used, but equations were added to arrive at these values; [b]data from Gu and Karthikeyan, 2005; [c] data from MINEQL+ database (Version 4.5, 2002); [d]equation and value from this study; [e]data from Coibion and Laszio, 1979; [f]data from Phreeqc database (Version 2.13.2-1727, 2007); [g]data from Werner et al. 2006.

Chapter 2

Geochemical Speciation Modeling of Tetracycline Sorption to K- and Ca-Smectites

2.1 Introduction

Antibiotics were major discoveries the 20th century that were enormously used in human and veterinary medicine. These medicines protect the lives of millions of humans and animals every year [8]. Later, antibiotics began to be used as feed supplements at subtherapeutic levels to enhance productively and profitability in the livestock industry. In 2010, the usage of antimicrobials in animal foods reached approximately 13.5 million kg [9].

Tetracycline is a broad-spectrum antibiotic that was discovered in early of 1950s [10]. The chemical structure of tetracycline consists of four fused cyclic rings substituted with functional groups, including dimethylammonium, tricarbonylmethane, and diketone (Figure1.1). In the aqueous phase, these functional groups gain or lose protons and causing tetracycline to form cations, anions, and zwitterions (neutral) depending on solution pH (Figure 1.2).

Tetracycline is used extensively in human and veterinary medicine since the effectively treat a variety of common infectious diseases. Tetracycline usage comprised 42% of the total

antimicrobials used in food animal production , 5,602,281 kg, in 2010 [9]. Since large fractions of tetracycline are not metabolized in the animals, massive amounts of tetracycline are excreted with animal manures, either as the original compound or as its bioactive metabolites [11, 12]. Thus, tetracycline and its derivatives are transferred to soil [13, 14], surface water [15–18], and groundwater [14, 19, 20].

In the environment, tetracycline speciation in aqueous solutions can be very complex. In the simplest case, tetracycline in pure water already contains four species, depending on pH (Figure 1.2). Typically, aqueous solutions will also contain a variety of metal cations, including Ca^{2+}, Mg^{2+}, and K^{+}. These cations may form complexes with tetracycline, thus reducing the activity of uncomplexed tetracycline and forming additional species that must be considered (Figure 1.3). The activities of metal ions may be altered by the presence of organic ligands that can complex the metals, or by sorption of the metals to solid phases such as clay minerals or organic matter. Such competitive complexation or sorption of metals will generally increase the activity of aqueous uncomplexed tetracycline. Finally, tetracycline itself may sorb to a variety of soil minerals (e.g., smectite clays, goethite, or magnetite) and to soil organic matter, thus decreasing tetracycline activity in solution.

In order to understand this complexity of tetracycline speciation in environmental waters, computational tools are needed to iteratively and quantitatively estimate the distribution of tetracycline among its many possible species. Phreeqc is a tool that was developed over 35 years ago and continues to be improved [27] for thermodynamic modeling of aqueous environmental systems. Phreeqc is a public-domain model that has already been integrated with saturated- and unsaturated-flow water transport modules [28], so it provides a framework for future reactive-transport modeling.

Thermodynamic modeling, as in Phreeqc, is very useful because even the most sophis-

ticated analytical procedures such as liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS), [29] only measures the total tetracycline concentration in solution rather than the concentrations of the component, (e.g. changed) tetracycline species, which may manifest different chemistries. Thermodynamic approaches allow a more powerful interpretation of already sophisticated analytical LC-MS-MS data by estimating the distribution of tetracycline among its possible species. Therefore, Phreeqc modeling is a method for extracting the most value from analytical data sets in order to better understand complexation, sorption, and bioavailability of antibiotics in the environment.

The objective of the current study was to develop a quantitative model for tetracycline speciation in complex systems. A central goal is to be able to predict tetracycline sorption to K- and Ca-clay minerals as a function of realistic geochemical variables. While many of the relevant chemical equilibria have already been described, several new thermodynamic relationships were fit to the available data in order to describe the competitive adsorption of tetracycline to K- and Ca-clay minerals. Finally, the fit of the parameters to several independent datasets was tested.

2.2 Methods

The thermodynamic parameters used in this study are listed in Table 1.1. Equations and parameters that were newly developed for this study are highlighted in bold. Werner et al. [3] measured tetracycline speciation and complexation by Ca^{2+}, and Mg^{2+} as functions of pH. Their parameters were selected for the present study because they form a self-consistent set.

2.2.1 Treatment of Cation-Exchange in Phreeqc

For modeling cation exchange on clay minerals, the study followed the general method of Appelo and Postma [6]. In this method, all cation exchange equilibria are referenced to exchange with Na^+. That is, a Na-saturated clay is chosen as the thermodynamic "zero", and the energetic state of all other cations in the exchange complex are relative to this "zero". Thus, the cation exchange parameters in Table 1.1 for cation I^{i+} are referenced to this equation:

$$Na^+ + 1/i \ . \ I - X_i \iff Na - X + 1/i \ . \ I^{i+} \tag{2.1}$$

The Phreeqc database compiles all such reactions as half-reactions of the form:

$$Na^+ \ + \ X^- = NaX \quad logk = 0 \tag{2.2}$$

where X^- is a site of cation exchange on the clay mineral. The $logk$ parameter in Phreeqc is the logarithm (base 10) of the equilibrium constant (K_{exch}) for this cation-exchange half-reaction. Since $logk = 0$, K_{exch} must equal 1, and so if any other cation-exchange half-reaction is added to that of Na^+, the K_{exch} and the $logk$ for the overall reaction will simply be the K_{exch} and the $logk$ for the non-Na^+ ion. Therefore, all cation exchange equilibria are referenced to exchange with Na^+.

To illustrate how parameters in the database work, considered the exchange of K^+ for Ca^{2+} on a clay mineral. The K^+ half-reaction (Table 1.1) is:

$$K^+ \ + \ X^- = KX \quad logk = 0.7 \tag{2.3}$$

If we multiply the previous reaction by 2, we will obtain the following equation:

$$2K^+ \ + \ 2X^- = 2KX \quad logk = 1.4 \tag{2.4}$$

Also, the Ca- exchange reaction in Phreeqc (Table 1.1) is:

$$Ca^{2+} \ + \ 2X^- = CaX_2 \quad logk = +0.8 \tag{2.5}$$

Reversing the previous reaction:

$$CaX_2 = Ca^{2+} \ + \ 2X^- \quad logk = -0.8 \tag{2.6}$$

If we combine reactions 2.4 and 2.6, we will get the following equation:

$$2K^+ \ + \ Ca - X_2 \longrightarrow 2KX \ + \ Ca^{2+} \quad logk = +0.6 \tag{2.7}$$

Therefore, since $[\triangle G = -RTlnk = -RT(2.3)logk]$ and $R = 8.3145\ J/K.mol$, $T = 298K$ hence $RT = 2.48\ kJ/mol$. Thus, $\triangle G = -(2.48)(2.3)(0.6) = -3.4\ kJ/mol$.

For the cation exchange half reaction that involves only one exchange site:

$$K^+ \ + \ 1/2Ca - X_2 \longrightarrow KX \ + \ 1/2Ca^{2+} \quad logk = +0.3 \tag{2.8}$$

So, $\triangle G = -(2.48)(2.3)(0.3) = -1.7\ kJ/mol$.

To check the $K^+$and Ca^{2+} parameters in Table 1.1, the competitive cation exchange between K^+ and Ca^{2+} was calculated at three different ionic strengths (Figure 2.1). The results were as expected, and these parameters have been used in many modeling studies of

cation exchange involving K^+ and Ca^{2+} [32–34].

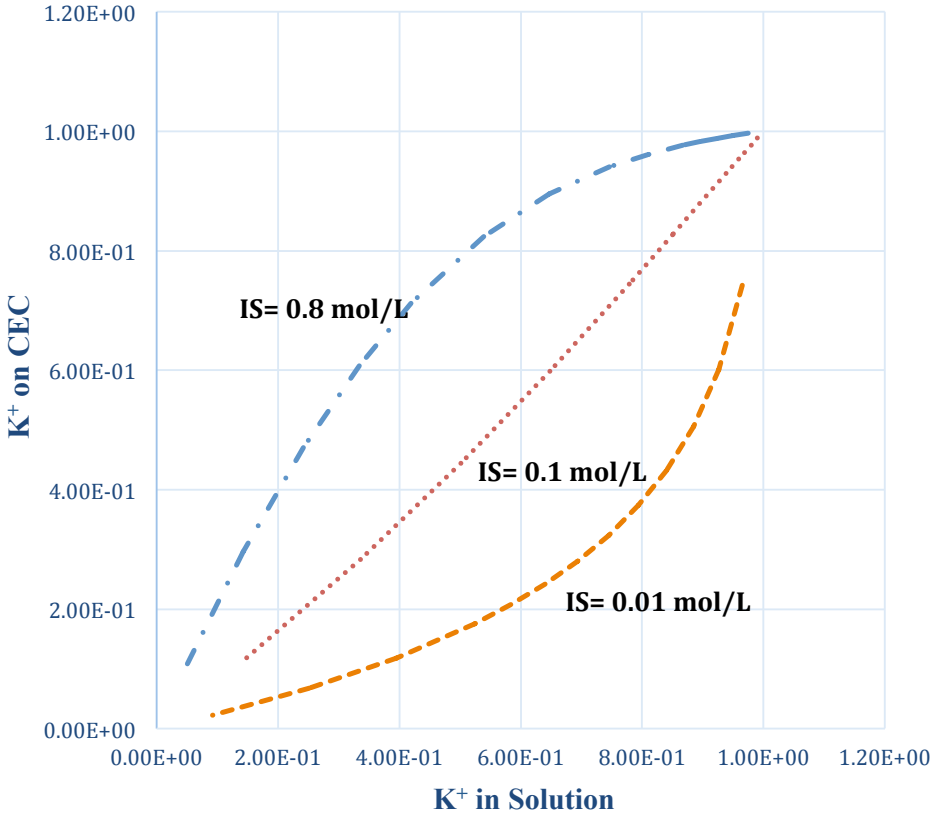

Figure 2.1 Calculated equilibria for K- Ca-exchange using Phreeqc and the cation exchange coefficients of Appelo and Postma [6] and Table 1.1 at three different ionic strengths. IS= ionic strength, and CEC= cation exchange capacity.

2.2.2 Experimental Data on Tetracycline Exchange with K^+ or Ca^{2+} on Clay Minerals

Yunjie Ding [7] measured the cation exchange equilibria of tetracycline with K- and Ca-smectites. This is a robust data set that provides sorption data for K^+, Ca^{2+}, and tetracycline as well as pH, the change in pH during experiment, and metal release from the clays.

Ding [7] measured metal release by K^+-clays and Ca^{2+}-clays during exchange reactions

with tetracycline. Such measurements were only possible for systems with no added background electrolyte (labelled 'no ionic strength' below), and could be useful for determining whether tetracycline adsorbs to clays in forms other than the H_4Tec^+ cation (Figure1.2). For example, when tetracycline sorbs to calcium smectite, there are at least three possible reaction stoichiometries. First, if two tetracycline molecules sorb to the clay and one Ca^{2+} ion appears in solution, then a reaction similar to

$$2H_4(Tec)^+_{(aq)} + CaX_2 = 2H_4(Tec)X + Ca^{2+} \tag{2.9}$$

is inferred. Second, if tetracycline sorbs to the clay but the amount of Ca^{2+} ions in solution remains constant, then a reaction like

$$H_3(Tec)^0_{(aq)} + CaX2 = [CaH_3(Tec)X_2] \tag{2.10}$$

could be inferred. Third, note that Figure1.3 shows that the $CaH_2(Tec)^+$ complex should be an important tetracycline species in calcium systems [3]. Sorption of that complex should result in one Ca^{2+} consumed from solution along with each two tetracycline sorbed, by the reaction:

$$2CaH_2(Tec)^+_{(aq)} + CaX_2 = 2[CaH_2(Tec)X] + Ca^{2+}_{(aq)} \tag{2.11}$$

Thus, the stoichiometry of changes in the inorganic cation concentration in solution as a function of tetracycline sorption can be used to constrain interpretations of the operant sorption mechanisms. Indeed, it may be feasible to use cation-release data and apply Equations 2.9 – 2.11 to estimate the speciation of tetracycline and calcium on the exchange complex.

In each initial tetracycline solution, the following equilibrium was established at the initial pH:

$$H_4(Tec)^+_{(aq)} \iff H_3(Tec)^o_{(aq)} + H^+_{(aq)} \iff H_2(Tec)^-_{(aq)} + 2H^+_{(aq)} \tag{2.12}$$

If the cationic species $H_4(Tec)^+$ sorbs to the clay and is removed from solution, and if we assume constant pH, then the above equilibrium will be re-established. If the amount of $H_4(Tec)^+$ removed from solution was C_i , then an amount of H^+ approximately equal to C_i will need to be removed from solution in order to re-establish the above equilibrium. Thus, proton consumption is an approximation of the amount of $H_4(Tec)^+$ sorption under a given condition, and should be reflected as a loss of protons from solution and an increase in the solution pH after tetracycline sorption by the clay.

2.3 Results and Discussion

2.3.1 Possible Impurities in the Clay or Deionized (DI) Water

To test the performance of the tetracycline parameters, the pH values for all initial solutions were calculated using Phreeqc. To do so, each initial concentration of tetracycline that was used by Ding [7] was equilibrated and the pH was calculated. The calculated pH was significantly lower than the observed pH, especially when the tetracycline concentrations were low. Ding used tetracycline HCl to make his initial solutions, so the following equations apply:

$$H_4TecCl \longrightarrow H_4Tec^+ + Cl^- \tag{2.13}$$

$$H_4Tec^+ \longrightarrow H^+ \ + \ H_3Tec^0 \tag{2.14}$$

Equation 2.14 shows that the solution pH should certainly be below 7 for all concentrations of tetracycline HCl in pure water. However, Ding observed many pH values above 8. A hypothesis that is consistent with Ding's data is that a basic impurity was present in all his systems. One possibility is carbonate impurities in the SWy-2 clay he used [35], and another possibility is impurities in the water. The deviations between observed and expected pH values were systematic and a good fit to all data was obtained by adding 0.08 $mmol/kgw$ of $NaHCO_3$ and 0.02 $mmol/kgw$ of Na_2CO_3. The calculated and observed pH values as a function of initial tetracycline concentrations are plotted in Figure 2.2. There is no evidence for the nature of the high pH contaminant in the clay or water used by Ding [7], but in order to fit pH properly, this buffer was used in all subsequent calculations.

Many complexation constants have been determined for tetracycline interactions with cationic metals, but the main focus of this study was to develop some new thermodynamic parameters for cation exchange reactions involving tetracycline. A provisional set of these parameters are listed and highlighted in Table 1.1.

One important parameter for clay minerals is the cation exchange capacity (CEC), the total quantity of cationic charge that can be reversibly sorbed per unit mass of clay. For the clay mineral SWy-2 used by Ding [7], the CEC is known to be 78 $cmol/kg$. However, sorption of tetracycline to both K-SWy-2 and Ca-SWy-2 with no other salt in the system showed sorption plateaus at $42 - 43$ $cmol$ of tetracycline per kg clay p.79 of [7]. A plausible reason for this discrepancy is explained by Ding p.88 of [7]:

"The basal spacing of smectites were 14.7 $\mathring{A}$ with tetracycline loadings < 135 $\mu mol/g$ ...

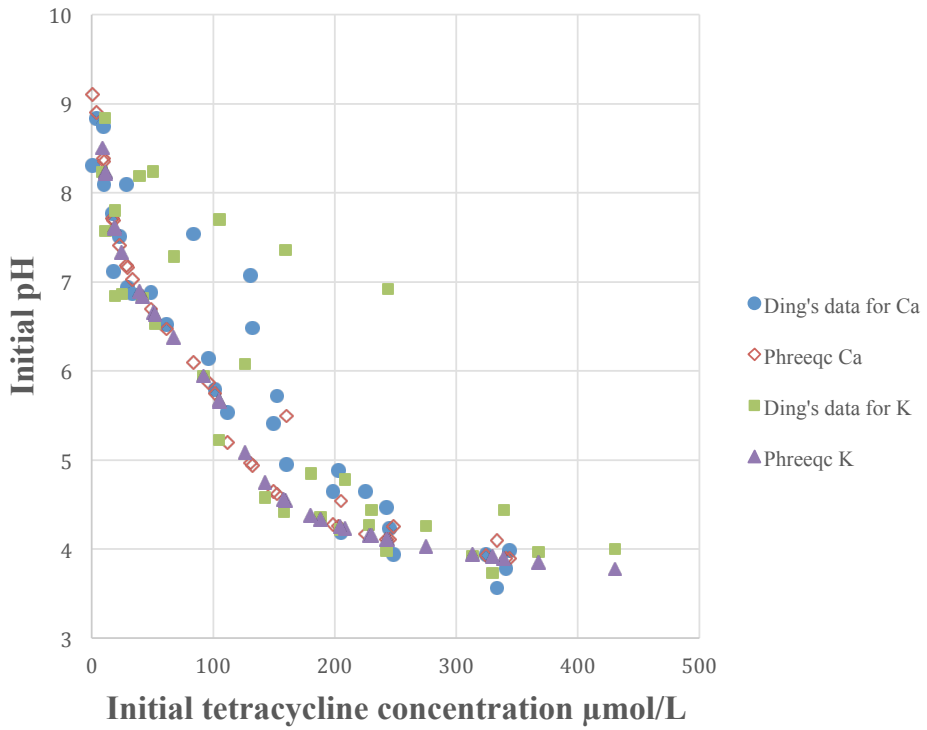

Figure 2.2 Comparison of modeled and experimental [7] pH values of initial tetracycline solution.

the distance between two adjacent clay sheets was 5.1 $\mathring{A}$ ... molecular dynamics simulation results indicate that at low loading rate ($< 135\ \mu mol/g$), tetracycline lays parallel to clay surfaces, ... When sorption approaches to 420 $\mu mol/g$, which is the sorption plateau in Figure III-2, the clay sheets expands to 7.5 $\mathring{A}$. At this distance tetracycline adapts a tilted position in clay interlayers. Molecular dynamic simulation results for sorption at 420 $\mu mol/g$ indicate that tetracycline molecules adapt vertically tilted position."

The present study also adopts the hypothesis that the clay interlayer "fills up" at $42-43$ $cmol(+)/kg$ at which point the interlayer is plausibly "full" and causes a sorption plateau. Further sorption of tetracycline is possible but requires the interlayer to rearrange so that tetracycline can adopt a tilted configuration. Realistically, loading of clay minerals in nature by tetracycline should be less than $42-43$ $cmol(+)/kg$, so confining our fitting efforts to this lower- tetracycline region of the sorption curve seems appropriate from an environmental,

practical standpoint. Ding [7] showed that tetracycline concentrations greater than 100 $\mu mol/L$ (44 $mg\ tetracycline/L$) were required to achieve the sorption plateau even in the absence of competing salts. For comparison, observed concentrations of tetracycline in liquid manures may approach 40 $mg\ tetracycline/L$ [36], but concentrations will generally be much more dilute in environmental waters or soil solutions.

One benefit of this approach is that such a model might fit all smectite clays and thus be widely applicable: All smectites have $CEC > 42 - 43\ cmol(+)/kg$ but all possess roughly the same interlayer surface area (about 750 m^2/g) [30], and so many smectites should display plateaus or inflections in their tetracycline sorption near $42 - 43\ cmol(+)/kg$. For example, Figueroa et al. [37] studied sorption of oxytetracycline and tetracycline to Na-montmorillonite. Their oxytetracycline sorption isotherm at pH 5.5 and 10 $mmol\ NaCl$ showed a plateau at about $440 - 480\ cmol(+)/kg$—their Fig. 7a labels that plateau as 44 cmol(+)/kg, but this seems to be in conflict with the reported K_d of 5500 $L/mol(+)$ (see their Fig. 3 at 10 $mmol\ NaCl$ and pH 5.5). At 0.1 $mmol/L$ tetracycline and with a clay of $CEC = 80\ cmol(+)/kg$, the K_d predicts sorption of 440 $mmol$ tetracycline per kg clay, exactly 10x the plateau pictured in their Fig. 7a. Another paper (Li et al., [38]) also commented that the sorption results of Figueroa et al. [37] seem low by a factor of about 10. Furthermore, the same authors (Li et al., [38]) observed an anomalous result that up to 42 $cmol(+)/kg$ of tetracycline could sorb to SWy-2 smectite without any significant change in the desorbed inorganic cations in solution. Again, this phenomenon and the high pH they observed, are indirect evidence of carbonate impurities in the SWy-2 smectite clay itself. Therefore, the present study fixed the quantity of cation exchange sites in the "exchange" module of Phreeqc at 0.42 moles of exchange sites per kg clay, and the study hypothesizes that this value should work for most smectite clays in the environment.

Aristilde et al. [39] observed maximum sorption of oxytetracycline (OTC^+) by smectite to be 44 $cmol(+)/kg$ even at OTC solution concentrations approaching 1 $mmol/L$ at pH 4 and in a background of 0.01 mol/L $NaNO_3$. On the basis of X-ray diffraction analysis and molecular simulations, they argued that OTC^+ was segregated into only half the smectite interlayers, with the other half of the interlayers filled with Na^+, a phenomenon known as demixing.

This study calculated $tetracycline/K^+$ exchange in 4 different ionic strength situations that also included several tetracycline concentrations and different pH values. A first situation was without ionic strength (no salt other than tetracycline HCl and sodium carbonate), while the remaining three cases added an additional 0.01 mol/L, 0.1 mol/L, and 0.8 mol/L as KCl.

The study adjusted the new thermodynamic parameters to fit Ding's results [7]. To do so, this study systematically examined several values of the $tetracycline/K^+$ exchange parameters before acceptable results were reached. Then, after a number of attempts, the research achieved a good fit for $tetracycline/K^+$ exchange.

Figure 2.3 shows the comparison between the Phreeqc predictions and Ding's data [7] at zero ionic strength for tetracycline sorption by K-smectite, and the result was fit very well. Note that Essington et al. [40] modeled the sorption of chlortetracycline (CTC) sorption by Na-smectite. They concluded that cation-exchange of CTC^+ for Na^+ was the dominant sorption process below pH 5, but that sorption above pH 5 was only slightly smaller in magnitude and needed to invoke strong sorption of the zwitterionic ($neutral\ CTC^0$) form of CTC. The present study suggests that interlayer sorption of the Na^+-CTC^0 complex is a species they could have considered.

Figure 2.4 compares the Phreeqc predictions with Ding's data [7] at 0.01 mol/L as KCl

for tetracycline sorption by K-smectite. The result was good, but the prediction of Phreeqc was slightly higher than Ding's data. Figure 2.5 shows the comparison between the Phreeqc predictions and Ding's data [7] at 0.1 mol/L as KCl ionic strength. In this case, tetracycline sorption by K-smectite was predicted very well. Figure 2.6 shows the comparison between Phreeqc prediction and Ding's data [7] at 0.8 mol/L as KCl ionic strength for tetracycline sorption by K-smectite. Again, the result was good, but the prediction of Phreeqc was slightly lower than Ding's data.

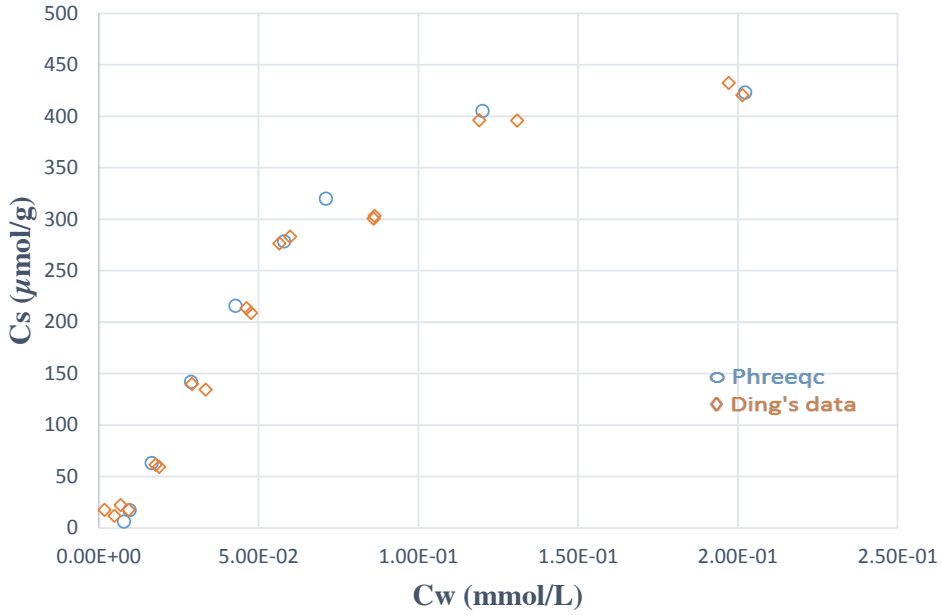

Figure 2.3 Sorption of tetracycline to K-smectite at zero ionic strength.

Next, the study attempted to create $tetracycline/Ca^{2+}$ exchange parameters that were consistent with the well-fitting $tetracycline/K^{+}$ parameters. For example, this study fixed the H_4Tec^{+}/K^{+} and H_4Tec^{+}/Ca^{2+} parameters at the same values that were effective in K-clay systems and only varied the exchange parameters for $Ca^{2+}/tetracycline$ complexes such as CaH_2Tec^{+}/Ca^{2+}.

Figure 2.7 shows the comparison between the Phreeqc predictions and Ding's data [7] at zero ionic strength for tetracycline sorption by Ca-smectite, and the model results fit

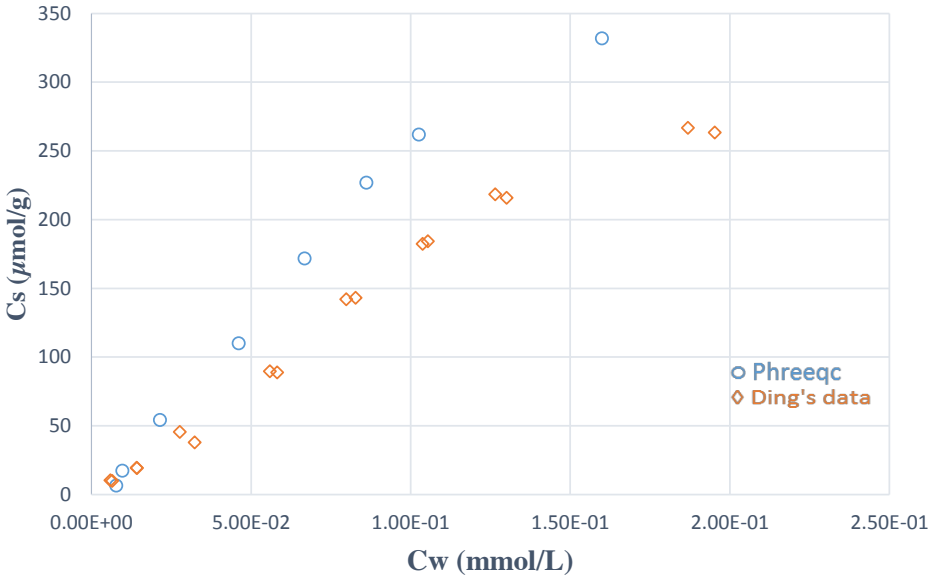

Figure 2.4 Sorption of tetracycline to K-smectite at 0.01 mol/L ionic strength.

experimental data very well. Figure 2.8 compares the Phreeqc predictions with Ding's data [7] at 0.01 mol/L as $CaCl_2$ for tetracycline sorption by Ca-smectite. Also, the agreement between model and experiment was very good. While Figures 2.7 and 2.8 make it tempting to believe that a good overall fit had been found using the modeling parameters compiled in Table 1.1, the results at higher ionic strength show this to be an illusion. Thus, great care must be advised when using goodness-of-fit as the main criterion for selecting thermodynamic modeling parameters. Figure 2.9 shows the comparison between the Phreeqc predictions and Ding's data [7] at 0.1 mol/L as $CaCl_2$ ionic strength, and this time, the Phreeqc prediction of tetracycline sorption was considerably higher than Ding had observed. Figure 2.10 shows the comparison between the Phreeqc prediction and Ding's data [7] at 0.8 mol/L as $CaCl_2$ ionic strength for tetracycline sorption by Ca-smectite. Again, the result did not fit because the prediction of Phreeqc was considerably higher than Ding's data.

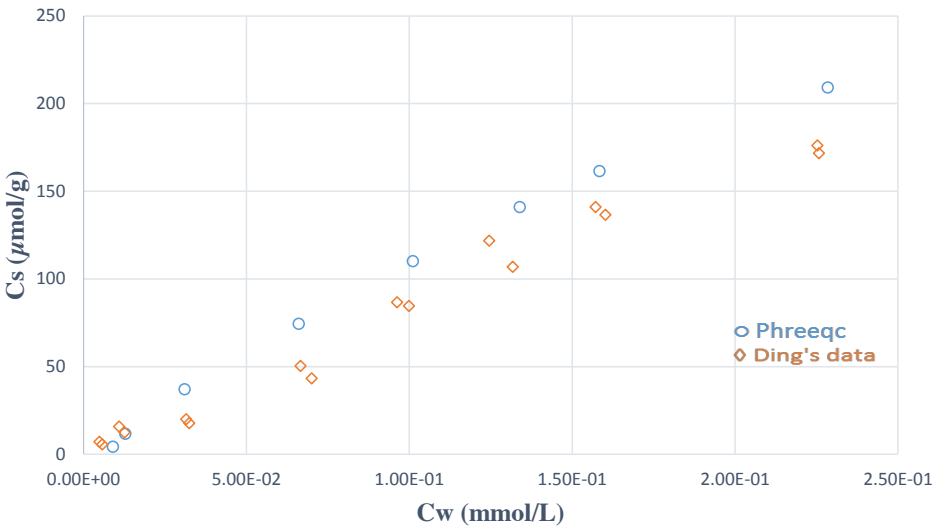

Figure 2.5 Sorption of tetracycline to K-smectite at 0.1 mol/L ionic strength.

2.3.2 Other Parameters that Could Fit Data

The good fits to the data in Figures 2.7 and 2.8 above seem to be for the "wrong reasons", namely that a too-large $\log k$ (7.0, Table 1.1) for the following reaction:

$$CaH_2(Tec)^+ + X^- = CaH_2(Tec)X \tag{2.15}$$

is compensating for a too-small $\log k$ (3.8, Table 1.1) for this reaction:

$$H_4(Tec)^+ + X^- = H_4(Tec)X \tag{2.16}$$

relative to Ca^{2+}. The very poor fits shown in Figures 2.9 and 2.10 make it clear that the $\log k$ for Eq. 2.15 was far too large, because the model predicted sorption that was far too strong (compared to experimental data) at high ionic strength.

Two questions then present themselves:

1) If $\log k = 7.0$ is much too large for Eq. 2.15, then what is a better value for modeling the data?

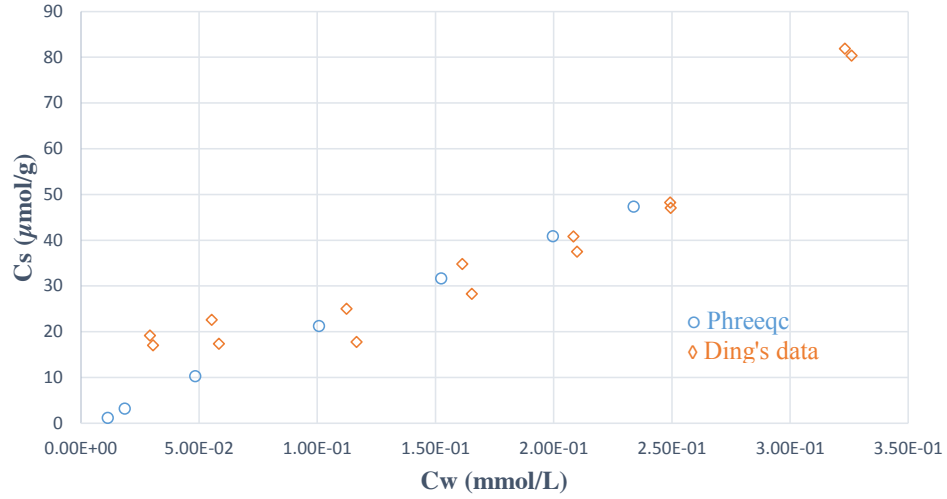

Figure 2.6 Sorption of tetracycline to K-smectite at 0.8 mol/L ionic strength.

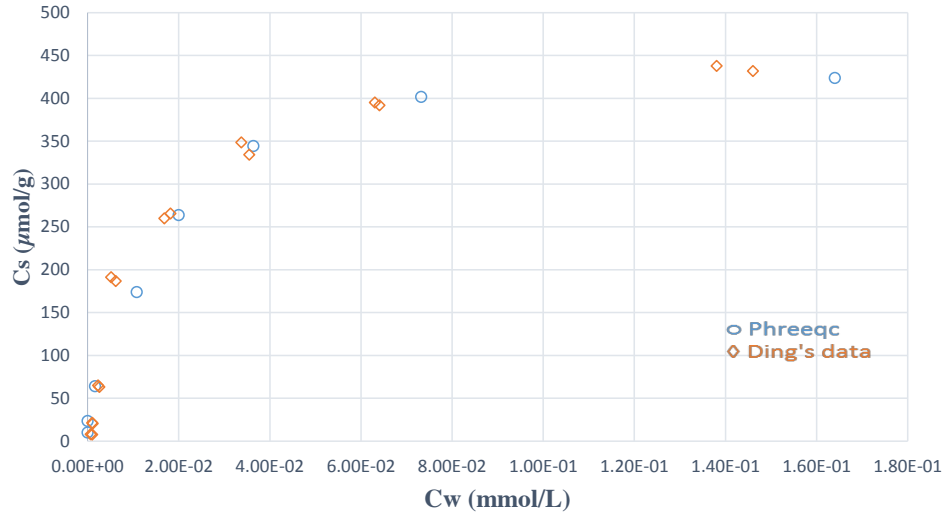

Figure 2.7 Sorption of tetracycline to Ca-smectite at zero ionic strength.

2) If $\log k = 3.8$ is too small for Eq. 2.16, relative to Ca^{2+}, then what is a better value for modeling the data?

At this point, it becomes very clear that Ding [7] showed excellent foresight in measuring tetracycline sorption across broad ranges of pH (Figure 2.2) and ionic strength. Figure 1.3 shows that the dominant cationic form of tetracycline in the pH region $6-8$ is $CaH_2(Tec)^+$, and Ding performed dozens of experiments at various ionic strengths in that pH range. These data at relatively high pH, and especially when ionic strength is also high, should be where

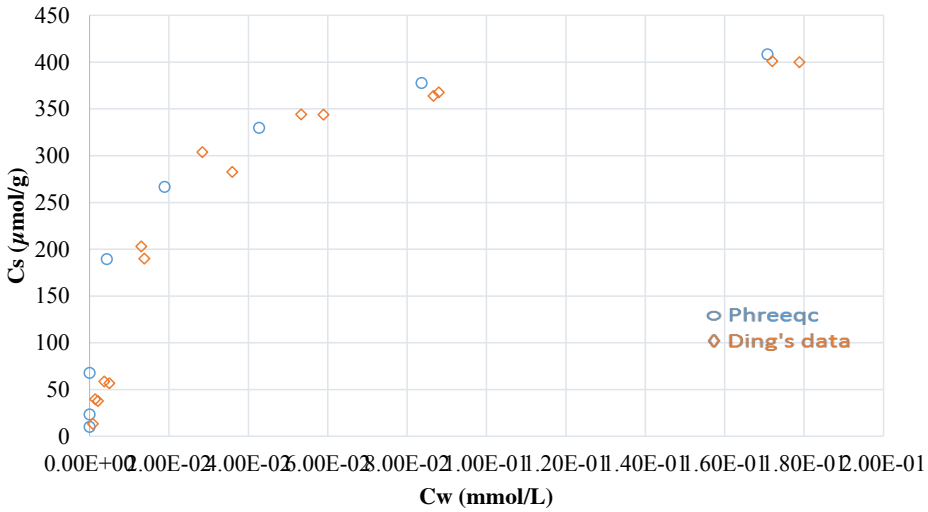

Figure 2.8 Sorption of tetracycline to Ca-smectite at 0.01 mol/L ionic strength.

Eq. 2.15 would be expected to be the dominant competitor in cation-exchange reactions with Ca^{2+}.

Conversely, Ding [7] also gathered dozens of datapoints at pH values below 5, often when the ionic strength was very low. These are the conditions under which $H_4(Tec)^+$ and therefore Eq. 2.16 ought to be the dominant competitor in cation-exchange reactions with Ca^{2+}. Thus, Ding's datasets provide optimism that parameters for Equations 2.15 and 2.16 can both be refined and also be consistent with the K-clay results.

Following this logic, the study searched for a $\log k$ value that allows Eq. 2.16 to reproduce the tetracycline-calcium competition for exchange sites fairly well at low ionic strength and low pH. The data point at farthest right in Figure 2.11 was predicted with $\log k = 5.5$ and corresponds (experimentally) to pH 4 to 5 and very low ionic strength. Thus, a $\log k$ of perhaps 6 might be a better estimate for the thermodynamics of Eq. 2.16. However, recall that in the case of $K^+/H_4(Tec)^+$ exchange, a $\log k = 3.8$ for Eq. 2.16 worked very well.

To find an approximate value for the thermodynamics of Eq. 2.15, we searched for good fits to data at high pH and high ionic strength. One example below with $\log k = 3.0$ for Eq.

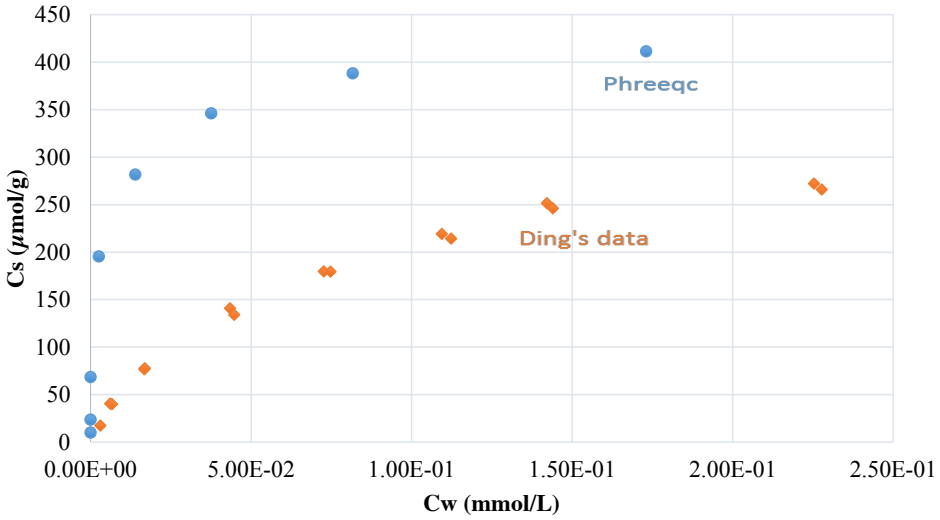

Figure 2.9 Sorption of tetracycline to Ca-smectite at 0.1 mol/L ionic strength.

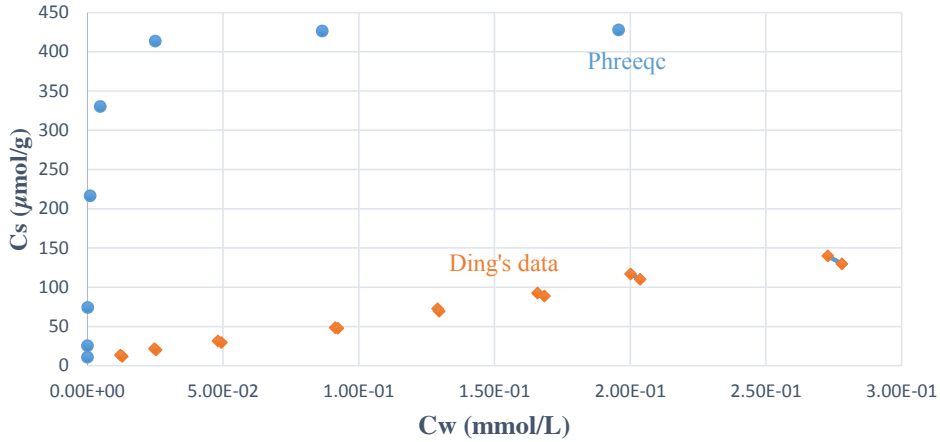

Figure 2.10 Sorption of tetracycline to Ca-smectite at 0.8 mol/L ionic strength.

2.15 shows good fits (especially for the higher-pH points at lower tetracycline concentrations) when ionic strength was either 0.1 mol/L (Figure 2.12) or 0.8 mol/L (Figure 2.13).

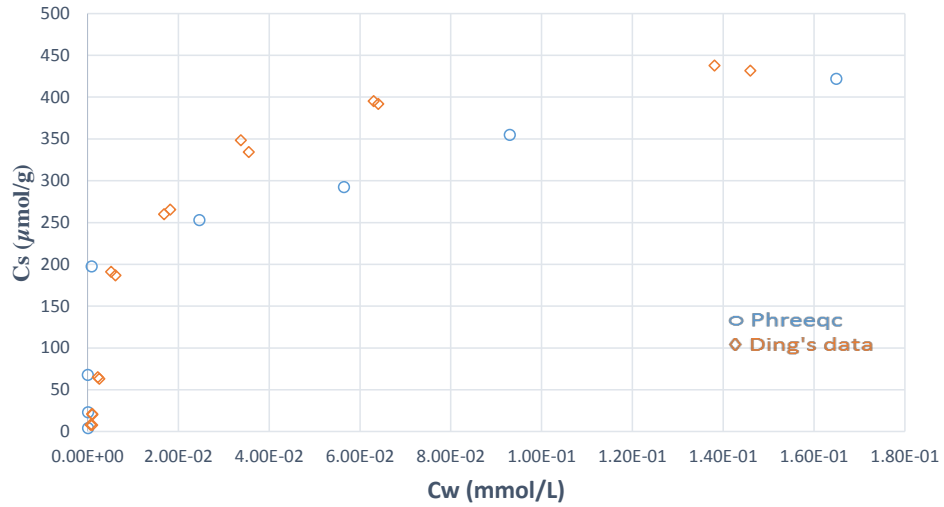

Figure 2.11 Modeled versus experimental [7] $Ca^{2+}/H_4(Tec)^+$ exchange when $\log k = 5.5$ for Eq. (2.16) and $\log k = 3.0$ for Eq. (2.15) at zero ionic strength.

2.4 Reconciling for Tetracycline Exchange with Both K- and Ca-Clay Minerals

The Phreeqc approach was able to model $K^+/H_4(Tec)^+$ exchange fairly well when $logk = 3.8$ for Eq. 2.16, while the $Ca^{2+}/H_4(Tec)^+$ exchange appeared to demand $logk = 6.0$ for the same equation in order to achieve a good fit. This is a significant problem that needs to be reconciled. If that could be done, then it seems plausible that the cation-exchange terms for K- and Ca-complexes could also be fit, and an overall set of self-consistent parameters could be achieved for general K-Ca-tetracycline-clay systems.

2.5 Path Forward for Future Research

The current research problem is that it seems impossible to simultaneously fit the cation exchange of tetracycline with both K^+ and Ca^{2+}. In particular, if tetracycline exchange with K^+ was modeled well, then tetracycline exchange against Ca^{2+} was too weak, espe-

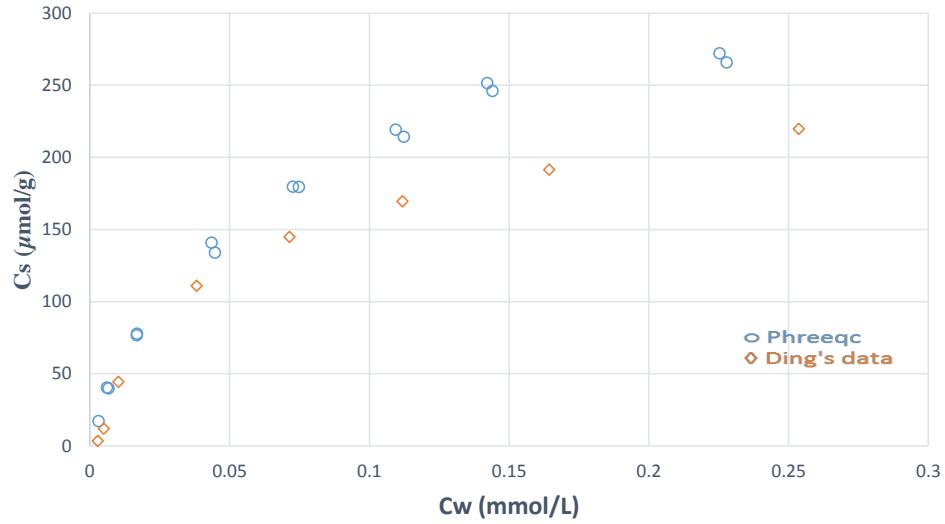

Figure 2.12 Modeled versus experimental [7] $Ca^{2+}/H_4(Tec)^+$ exchange when $\log k = 5.5$ for Eq. (2.16) and $\log k = 3.0$ for Eq. (2.15) at 0.1 mol/L ionic strength.

cially at high ionic strengths. In terms of Gibbs free energy [31], it seems that $\triangle G$ for $tetracycline/Ca^{2+}$ exchange needs to be more negative. Until now, the study has assumed that the literature values for K^+/Ca^{2+} exchange that are embodied in Phreeqc [6] were adequate. Indeed, they do seem plausible (Figure 2.1) . Equation 2.8 shows that $\triangle G$ of K- Ca-exchange is about -1.6 kJ/mol. Figure 2.14a shows this relationship graphically. Reviews of the literature [32], show that the previously estimated values for K- Ca-exchange range from +2 to -14 kJ/mol. The "best" value from this range should be the subject of a thorough literature review, but the "best" value seems likely to be more negative than -1.6 kJ/mol.

Suppose this "best" value is -4.6 kJ/mol. Then, K^+/Ca^{2+} exchange would look like Figure 2.14b. Suppose also that one had created a perfect parameter for $K/tetracycline$ exchange of $\triangle G$ for $H_4(Tec)^+/K^+$ that was -17.7 kJ/mol (Figure 2.14). Note that if the study desires to simultaneously:

a) Increase the magnitude of $\triangle G$ for K^+/Ca^{2+} exchange from -1.6 kJ/mol (Figure

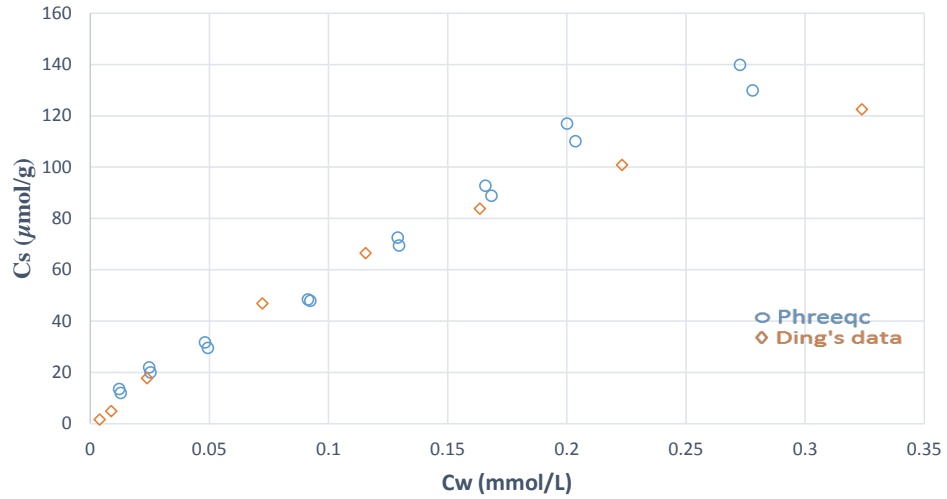

Figure 2.13 Modeled versus experimental [7] $Ca^{2+}/H_4(Tec)^+$ exchange when $\log k = 5.5$ for Eq. (2.16) and $\log k = 3.0$ for Eq. (2.15) at 0.1 mol/L ionic strength.

2.14a) to -4.6 kJ/mol (Figure 2.14b) to reflect the "best" value from the literature [32].

b) Maintain the same value of $\triangle G$ for $H_4(Tec)^+/K^+$, which is -17.7 kJ/mol [Both Figures 2.14a and 2.14b].

Then, observe that the value of $\triangle G$ for $H4(Tec)+/Ca^{2+}$ has increased from -19.3 kJ/mol (Figure2.14a) to -22.3 kJ/mol (Figure 2.14b). Therefore, making the $\triangle G$ for K^+/Ca^{2+} exchange more negative and more favorable, for a given value of $\triangle G$ for $H_4(Tec)^+/K^+$, has the effect of also making the value of $\triangle G$ for $H_4(Tec)^+/Ca^{2+}$ more negative and more favorable. This change in the value of $\triangle G$ for $H_4(Tec)^+/Ca^{2+}$ is in the right direction to enable tetracycline to compete more effectively against Ca^{2+}. The study hypothesizes that such a change will allow the cation exchange of tetracycline with both K^+ and Ca^{2+} to be fit simultaneously.

An alternative strategy to looking through the literature for a "best" value of $\triangle G$ for K^+/Ca^{2+} exchange would be to ask what value of $\triangle G$ for $H_4(Tec)^+/Ca^{2+}$ might be required to fit the data. In this case, a tentative answer is provided above, when the study suggested that $logk$ near 6 might be required to make Eq. 2.16 work for Ca-systems. This

$logk$ = 6 corresponds to $\triangle G$ = -34.5 kJ/mol for Eq. 2.16 in order to enable accurate modeling of $H_4(Tec)^+/Ca^{+2}$

In terms of Figure 2.14b, if $\triangle G$ for $H_4(Tec)^+$ (Eq. 2.11) were -34.5 kJ/mol and $\triangle G$ for $H_4(Tec)^+/K^+$ were still fixed at -17.7 kJ/mol (Figure 2.14a and b), then the overall energy-level diagram would be constrained to look like Figure 2.15.

Essington et al. [40] measured and attempted to model chlortetracycline (CTC) sorption by Na-smectite. They concluded that cation-exchange was the dominant process below pH 5 and their fits to experiment resulted in the following reactions:

$$H^+_{(aq)} + X^- = XH \quad logk = -2.2 \tag{2.17}$$

$$H^+_{(aq)} + XNa = XH + Na^+_{(aq)} \quad logk = -4.6 \tag{2.18}$$

$$XH + CTC^+_{(aq)} = XCTC + H^+_{(aq)} \quad logk = +8.75 \tag{2.19}$$

Adding Equations 2.17 and 2.19 yields

$$X^- + CTC^+_{(aq)} = XCTC \quad logk = +6.55 \tag{2.20}$$

and both the form of Eq. 2.20 and the log k of 6.55 correspond very well to the present study's suggestion of log k = 6 for Eq. 2.16 above.

Alternatively, adding Equations 2.18 and 2.19 yields

$$XNa + CTC^+_{(aq)} = XCTC + Na^+_{(aq)} \quad logk = +4.15 \tag{2.21}$$

which relates the exchange of CTC^{+} to that of Na^{+}, and provides an estimate for log K following the convention of Appelo and Postma [6].

For future work, this study hypothesizes that the $tetracycline/K^{+}/Ca^{2+}$ exchange parameters shown in Figure 2.15b, with some refinement, should allow the approximately self-consistent modeling of tetracycline cation exchange reactions with a single set of parameters. This would enable complete speciation of tetracycline in complex mixtures of multiple inorganic cations and clay minerals. Furthermore, adding in the bacterial distribution method from Chapter 1 would allow modeling of bacterial uptake and the induction of antibiotic resistance in these complex mixtures.

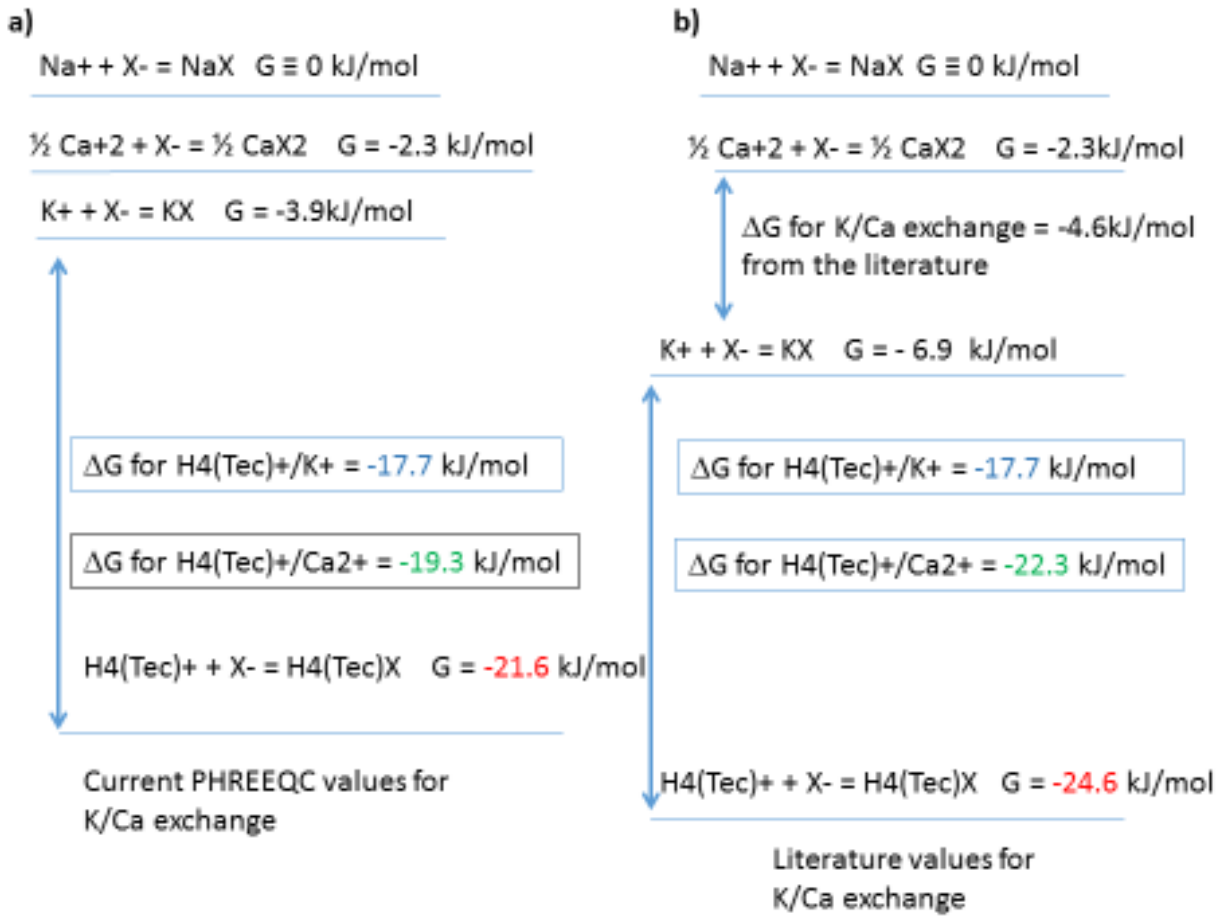

Figure 2.14 Free energy diagram for a subset of cation exchange reactions in Phreeqc. Current Phreeqc parameters (a) are shown on the left, while a hypothetical future parameters set (b) is shown on the right.

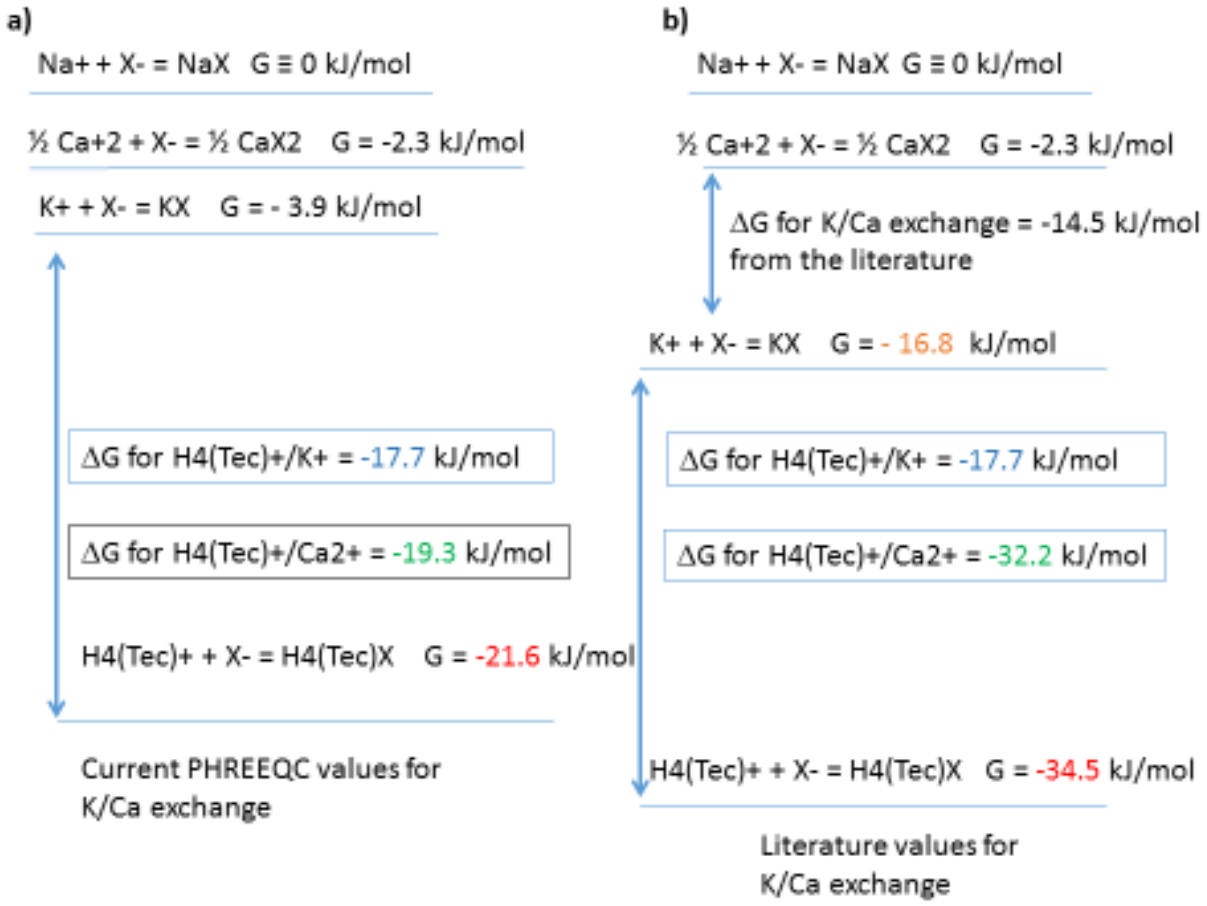

Figure 2.15 Free energy diagram for a subset of cation exchange reactions in Phreeqc. Current Phreeqc parameters (a) are shown on the left, while a hypothetical future parameters set (b) is shown on the right.

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