SORPTION OF TETRACYCLINES IN DESERT-SOIL ENVIRONMENTS

By

Mohammed Ahmed Alsanad

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ABSTRACT

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The broad-spectrum antibiotic tetracycline is used extensively for human and animal health, but causes unintended environmental consequences. Significant amounts of tetracycline are excreted with animal manures and can pollute soil, surface water, and groundwater. The overall objective of this dissertation is to combine experimental and modeling work to determine the environmental chemistry of tetracyclines in desert soils.

Some previous studied observed that a) when Ca^{2+} -to-tetracycline ratios were large, then $Ca_2H(Tec)^{2+}$ species dominate in the solution above pH 5, and b) at low concentration of cation and high pH, Ca^{2+} - and Mg^{2+} -clays sorbed significantly large amounts of oxytetracycline. Since Saudi Arabian topsoils can have pH near 7.5 along with large Ca^{2+} concentrations, these conditions together may enable relatively strong sorption of cationic Ca^{2+} -tetracycline complexes by cation exchange capacities (CEC) of clay minerals. Thus, the objective of current study was to test this hypothesis. To do so, soil samples were collected from the Agricultural and Veterinary Training and Research Station at King Faisal University, Al-Ahsa, Saudi Arabia. Three soils with relatively high clay contents were selected, and sorption isotherms were measured by using liquid chromatography/tandem mass spectrometry (LC-MS/MS) instrument to quantify oxytetracycline (OTC) concentrations. The results of this study showed that oxytetracycline sorption was significant at pH 7.5 for all three soils, and the hypothesis of this study - that desert soils at pH 7.5 may adsorb reasonably large amounts of oxytetracycline - was supported.

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 to understand and predict partitioning of tetracycline into its various species. The objective of this study was to use many experimental data sets to create new thermodynamic parameters using Phreeqc for modeling the sorption speciation of tetracyclines. Since clay minerals are important sorbents, cation exchange parameters were developed for tetracycline and its K^+ - and Ca^{2+} -complexes for better understanding of that very complicated system. A self-consistent set of parameters was derived that enabled tetracycline cation-exchange to be modeled in both K^+ - and Ca^{2+} -systems over a range in pH from 4 to 8.

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Chapter 1

Tetracycline Sorption in Saudi Arabian Soils

1.1 Introduction

Tetracycline antibiotics are supplied to animal feeds for multiple purposes such as protection and treatment from many diseases and to enhance the growth as well. Up to the present time, antibiotic resistance is still to form fears into our environment system which requires further particular research [8]. In 1950s, tetracycline antibiotics were discovered [9]. A recent report showed that 51 tons of tetracyclines are consumed every day in the U.S. only, and around 80% of tetracyclines antibiotics are utilized in the livestocks while a small amount of about 20% is utilized for people and another purposes [10]. And the tetracycline antibiotic chemical structure involves from four bonded cyclic rings substituted with functional groups, containing dimethylammonium, tricarbonylmethane, and diketone (Figure 1.1). Several studies and research were notified that antibiotics uses in agricultural practices have a danger impact to the human health [11–13].

Since great portions of tetracycline antibiotic are not digested entirely within the animals' bodies, thus quantities of tetracyclines antibiotics are discharged with animal manures to the environment, such as a parent compound or like their bioactive metabolites. Hence, tetracyclines antibiotics and their secondary forms are transported to soils, surface waters, and groundwaters [14–16]. Paul Ebner reported some antibiotics concentrations ranges which found in the environment [17]. For example, concentrations of tetracycline antibiotic in animal manure were ranging from $0.04 \ mg/kg$ to $24.0 \ mg/kg$, and tetracycline concentrations in soil were ranging from $86 \ \mu g/kg$ to $172 \ \mu g/kg$ while tetracycline concentration in surface water was $0.11 \ \mu g/L$ [17].

As we know that the sorption dominates the fate of substances and compounds in clays and soils, therefore this process must be considered. The accumulation and stability of any substance in soil or clay surfaces relies on soil properties, such as soil pH, clay content, clay minerals, and cation exchange capacity [18, 19]. Further, the study of Sarmah et al. indicated that physical and chemical parameters such as chemical structure, solubility in water, and compound species have great impacts on sorption mechanism [20]. Many years ago, exploring the fate of tetracyclines antibiotics in the environment started and, it remains until nowadays in wide parts of the world.

Since pH of solution controls speciation of ionable compounds, tetracycline functional groups usually turn into ionized and lead tetracycline to form cationic, zwitterionic (neutral), or anionic species in the solution phase, (Figure 1.2).

Generally, solution phase will also consist of many different cationic metals, such as Ca^{2+} , Mg^{2+} , and K^+ which will combine with the tetracycline compound. Hence, this will decrease the activity of uncomplexed tetracycline and making new varied species which should be estimated (Figure 1.3).

For soil systems that contain swelling clay minerals, it has long been known that low pH enhances the formation of cationic tetracyclines that sorb quite strongly to the negatively charged clay surfaces [21–24].

Early work [25] indicated another mechanism for tetracycline sorption, because tetracycline was found intercalated into clay interlayers at both pH 5 and 8.7, and spectroscopic evidence showed Ca-tetracycline complexation. These authors concluded that tetracycline could sorb by either cation-exchange of the $H_4(Tec)^+$ species, or through sorption of unknown Ca-tetracycline complexes. This complexation hypothesis was further supported [26], in that Ca^{2+} smectite sorb more tetracycline than that Na^+ smectite at given pH and extraction of all sorbed cations implied Ca-tetracycline complexes had been sorbed to the clay. Further work [27] showed that Ca^{2+} smectites sorbed more tetracycline than Na^+ smectites over the entire pH range from 7 to 9.

At moderate to neutral pH, strong sorption is also sometimes observed [21,24], although such sorption is often attributed to tetracycline interactions with soil organic matter [24,26] or oxide mineral surfaces [28–30]. However, in 2013, Parolo et al. studied sorption of tetracycline to montmorillonite in 0.01 M NaCl, and they discovered that at pH 7 to 9, Ca^{2+} clays sorbed large amounts of oxytetracycline (50 cmol/kg) [7]. The authors hypothesize that Ca^{2+} -oxytetracycline complexes are involved. Also, Parolo et al. observed that when $[Ca^{2+}]$ = 15.2 mM and [tetracycline] = 0.76 mM (300 ppm), then $Ca^{2H}(Tec)^{2+}$ species dominate in the solution above pH 5 [7]. This is a plausible species to participate in cation exchange.

Further, in 2016, Aristilde et al. their study showed that at low concentration of divalent cation and high pH, Ca^{2+} - and Mg^{2+} -clays sorbed significantly larger amounts of oxytetra-cycline than Na^+ -clays [31]. This implies Ca^{2+} - and Mg^{2+} -oxytetracycline complexes are involved.

In studying tetracycline sorption to montmorillonite Xu et al. [32] observed that there were two adsorption maxima, one below pH 4 and another at pH 7.7. Since their bulk clay contained a variety of cations including Ca^{2+} , a reasonable hypothesis for explaining their

sorption maximum at pH 7.7 is the sorption of cationic Ca-tetracycline complex by the clay.

At low pH values, Ding [6] observed tetracycline sorption to montmorillonite clay of up to 19% tetracycline by weight. For palygorskite which has a smectite like composition but does not swell, Chang et al. [33] measured a pH maximum in sorption at about pH 8.7, at which the palygorskite sorbed 10% tetracycline by weight.

Relevant, strongly-sorbing clay minerals are common in arid environments. For example, the general area surrounding Al-Ahsa, Saudi Arabia is rich in smectites and especially palygorskite [34] which has a high surface area [35] as smectite but it doesn't swell as a smectite clay. However, it can hold cations in the channels (Channels size $\approx 6.4 \times 3.7$ Å [36]; S.A. $\approx 150 \ m^2/g$ [37]; CEC of palygorskite $\approx 20 \ meq/100g$ [36]) and water [35]. Reasonably, palygorskite can hold tetracycline.

Furthermore, Al-Hawas [1] extensively characterized the clay mineralogies of eight soils near the King Faisal University Research Station in Al-Ahsa. His results are in agreement, and indicate (Table 1.1) that the general clay mineralogy of the area is;

palygorskite > smectite > kaolinite \approx calcite.

Thus, Saudi Arabian topsoils, including those in Al-Ahsa city, can have substantial clay contents that include smectite and palygorskite [1,34]. In addition, pH values in such soils are generally alkaline, and the soils are often rich in divalent cations such as Ca^{2+} . Understanding the sorption of veterinary antibiotics to such soils may be important for protecting environmental quality. For example, there is a large, commercial dairy farm immediately adjacent to the King Faisal University Research Station. The main hypothesis of this study is that desert-soil conditions (clays, pH, and soluble cations) may combine to enable relatively strong sorption of cationic Ca-oxytetracycline complexes by the *CECs* of clay minerals [7,31]. This study could locate no references to previous work that measured sorption of tetracy-

Soil Classification Estimated mineral [*] content (%) of the clay fraction							
	S	С	Р	Κ	Ι	Ca	Q
Typic Torripsamment	27	8	21	16	13	14	1
Gypsic Haplosalid	10	0	27	10	3	39	11
Typic Torrifluvent		12	56	18	6	2	1
Lithic Haplogypsid		2	49	9	15	23	1
Leptic Haplogypsid	33	11	39	8	5	2	2
Typic Torriorthent		0	9	6	7	4	1
Anthropic Torrifluvent	0	5	55	11	28	0	1
Cambic Gypsiorthid	4	2	55	3	9	26	1

Table 1.1 Mineralogies of the clay fractions found in eight topsoils of Al-Ahsa city near the King Faisal University research station [1].

*S=smectite, C=chlorite, P=palygorskite, K=kaolinite, I=illite, Ca=calcite, Q=quartz.

clines to desert soils. Indeed, there may be tacit assumptions that sorption would be low under such high-pH conditions [21–23].

Therefore, the objective of this study was to test this hypothesis by quantifying sorption to some desert-region topsoils.



Figure 1.1 Tetracycline chemical structure involves of four bonded cyclic rings, and three different pKa values [2].



Figure 1.2 Tetracycline species distribution in pure water as a function of solution pH calculated using the Phreeqc model [3].



Figure 1.3 Dominant species of tetracycline as a function of pH and Ca^{2+} concentration (mol/L) in aqueous solution [4]; L = tetracycline.

1.2 Materials and Methods

1.2.1 Study Area and Soil Characterization

In this current research, in August of 2016, the soil samples were gathered from the Research Station at King Faisal University which located in Al-Ahsa city at Al-hofuf district, in the east of Saudi Arabia (Figure 1.4). Typically, the weather in the country is desert climate which is cold and rainy in the winter while it is very hot in the summer time [38]. The maximum temperature in August often reaches 44.5°C (112.1°F) while the relative humidity (RH) often exceeds 90% at the same period [39]. Figure 1.5 shows soil samples locations of this study in the Agricultural and Veterinary Training and Research Station, Al-Ahsa city. The soil samples of this research were air dried at the room temperature for around 24 hours. After that, the soil samples were sieved using 2 mm screen. Stones and coarse roots were discarded, and comprised less than 5% of soil volume.

Saturated soil paste was prepared for each soil sample, 250 grams of soil sample was taken and then added DI water to the sample until it reached the saturation point. Then, the soil paste left for 24 hours [40]. After that, the soil paste was extracted using vacuum pump for 3 hours hence the liquid phase of soil sample was collected in plastic vial and stored in the refrigerator. The physio-chemical characterizations of soil measured via standard methods [41]. The particle size distribution (PSD) of soil was measured by hydrometer method [42]. The soil pH was measured by a pH meter while the electrical conductivity (EC) was measured by EC meter [40]. Ca^{2+} and Mg^{2+} cations measured via titration (by volumetric analysis using EDTA (p.44 of ref. [40]) while Na^+ and K^+ measured via Photometer Flame [40]. Also, CO_3 and HCO_3 ions were measured via titration with H_2SO_4 [43] while Cl^- ion was titrated with $AgNO_3$ [40]. The calcimeter method was used to measure $CaCO_3$ content [44].



Figure 1.4 Map of the study area which created using the Google Maps, and the blue balloon refers to research station at King Faisal University.

All these measurements were done in King Faisal University Labs. Table 1.2 and Table 1.3 show the physical and chemical characterizations of the soil samples.

1.2.2 Facts about the Agricultural and Veterinary Training and Research Station at KFU

It was established in 1977, and It is located away 15 km from King Faisal University campus. Research Station land area is 6 km^2 (600 hectares). Its soils are classified as Aridisols and Entisols. Wind-blown erosion is very active in this area [45].

Sample #	Depth (cm)	Clay $(\%)$	Soil Texture		
4	0-30	60	Clay		
19	0-30	32	Sandy Clay Loam		
23	0-30	18	Sandy Loam		
29	0-30	8	Sand		
1-3	0-30	8	Sandy Loam		
8-19	0-30	11	Sand		
21	0-30	8	Sand		
4-14	0-30	20	Sandy Clay Loam		
6-24	0-30	6	Sand		
28	0-30	14	Loamy Sand		
26	0-30	10	Sand		
20	0-30	8	Loamy Sand		
13	0-30	12	Sandy Loam		
30	0-30	6	Loamy Sand		
3	0-30	8	Sandy Loam		
47	0-30	6	Sand		
40	0-30	8	Loamy Sand		
33	0-30	10	Sand		
32	0-30	10	Sand		
1	0-30	10	Sand		

Table 1.2 Physical characterizations of all soil samples

$\mathrm{Sample}\#$	$Ca^{2+} * (meq/L)$	$Na^+ * (meq/L)$	$CaCO_3~(\%)$	pH	Total OC (%)
4	84.13	151.95	19.23	7.4	0.61
19	72.77	147.34	2.96	7.5	0.49
23	189.97	189.06	5.92	7.4	0.26
29	16.97	19.32	5.28	8	0.06
1-3	91.03	87.83	13.95	7.4	0.35
8-19	12.8	1.32	5.92	8.2	0.02
21	17.03	30.08	5.71	7.9	0.01
4-14	40.4	118.58	9.93	7.1	0.36
6-24	4.27	5.71	4.65	8.1	0.41
28	36.13	86.07	7.19	7.4	0.15
26	56.9	37.33	7.78	7.9	0.18
20	16.67	2.42	2.62	7.9	0.84
13	224.93	210.14	12.47	7.4	0.5
30	39.1	54.9	10.36	7.7	0.32
3	100.33	159.2	21.56	7.7	0.41
47	4.17	0.66	4.44	7	0.01
40	25.17	32.93	4.44	7.5	0.27
33	16.07	4.17	6.13	7.8	0.02
32	9.47	2.2	4.31	7.9	0.02
1	15.23	5.05	10.15	7.9	0.19

Table 1.3 Chemical characterizations or properties of all collected desert soil samples

 * Concentration or amount as measured in the solution extracted from the saturated paste.



Figure 1.5 Locations map of all soil samples in the Agricultural and Veterinary Training and research station at KFU which created using Google Maps.

1.2.3 Oxytetracycline Sorption to Soils 23 and 4

Two soils, designated as samples 23 and 4 (Tables 1.1 and 1.2), were chosen for initial study. Given the study hypothesis that Ca^{2+} oxytetracycline complexes are strongly sorbed to swelling clays [7,31], the soils were chosen because of their relatively high concentrations of both clay and extractible calcium. Approximately 0.5 g of soil was carefully weighed into glass vials.

The study prepared stock solutions of 50 mg/L oxytetracycline (Sigma oxytetracycline HCl, minimum 95% HPLC), 1.00 mol/L CaCl2, and 1.00 mol/L NaCl. The oxytetracycline stock solution was made freshly each day. Stock solutions were then combined (salt solution first and then oxytetracycline) to make working solutions (25 mL each) at concentrations of

200, 400, 600, 800, and 1000 μ g/L oxytetracycline in salt solutions that had concentrations of approximately pure water (no added salt), 0.01, or 0.1 mol/L. Each working solution was made in duplicate for each soil; for a given soil, one set of 10 working solutions was prepared at each concentration of each cation, with controls at each oxytetracycline concentration and each concentration of added salt. Then, each working solution was added to the appropriate vial of soil. The soil itself added both Ca^{2+} and Na^+ to each solution (Table 1.3). Thus, 0.5 g of soil 23 added 2.5 mmol Ca^{2+}/L and 4 mmol Na^+/L to each working solution, while 0.5 g of soil 4 added 3.6 mmol Ca^{2+}/L and 12 mmol Na^+/L to each working solution. Then, the study samples were covered with aluminum foil to exclude light and were put on a horizontal shaker for around 24 hours. After that, the samples were centrifuged for 15 minutes at 3500 rpm. Then, about 1 mL of the solution phase was taken from each sample.

Finally, the samples were analyzed using liquid chromatography/tandem mass spectrometry (LC-MS/MS) with a SCIEX API 3200 instrument. An aliquot of the centrifuged supernatant was used to measure the pH; pH values were typically in the range of 7.4 to 8.

1.2.4 Oxytetracycline Sorption to Soil 19

A third soil, designated sample 19 (Tables 1.1 and 1.2), was also studied. The soil was chosen because of its relatively high concentrations of both clay and extractible calcium. Approximately 0.5 or 0.2 g of soil was carefully weighed into glass vials.

In this case, experiments were conducted only in the absence of added salt. The study prepared stock solution of 50 mg/L oxytetracycline and working solutions (25 mL each); concentrations of 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 μ g/L, with controls at each concentration. Then, each working solution was added to the appropriate vial of soil. The soil itself added both Ca^{2+} and Na^+ to each solution (Table 1.3). Thus, 0.5 g of soil 19 added 1.3 mmol Ca^{2+}/L and 5 mmol Na^+/L to each working solution, while 0.2 g of soil 19 added 0.5 mmol Ca^{2+}/L and 2 mmol Na^+/L to each working solution. Then, the study samples were covered with aluminum foil to exclude light and put on a horizontal shaker for around 24 hours. After that, the samples were centrifuged for 15 minutes at 3500 rpm. Then about 1 mL of solution phase was taken from each sample and analyzed using a SCIEX API 3200 LC-MS/MS. Again, pH was determined using an aliquot of the centrifuged supernatant and pH values were typically in the range of 7.4 to 8.

1.3 Results and Discussion

1.3.1 Effects of Background Cations on Oxytetracycline Sorption

This research studied oxytetracycline sorption to two desert soils from background solutions containing Ca^{2+} or Na^{+} at three different concentrations. For example, Figure 1.6 shows oxytetracycline sorption to soil 23 after adding $CaCl_2$ concentrations of zero, 0.01, and 0.1 mol/L and also several oxytetracycline concentrations (ranging from 200 μ g/L to 1000 μ g/L).

First, sorption was strong with no added Ca^{2+} , and then sorption increased somewhat further in the presence of 0.01 mol/L added $CaCl_2$. These data seem to support the hypotheses that Ca^{2+} enhances oxytetracycline sorption to clayey soils at high pH. That is, Table 1.3 shows that soil 23 contains 95 mmol/L water-extractable Ca^{2+} in its saturated paste (250/65= 3.85 g soil/mL extract); under the experimental conditions with 0.5 g soil suspended in 5 mL of aqueous solution (0.1 g/mL), the water-extracted Ca^{2+} would be approximately 0.0025 mol/L in the experimental suspension with no added Ca^{2+} . According to the working hypothesis of this study, this amount of solution-phase Ca^{2+} was apparently enough to result in many cationic Ca-oxytetracycline complexes that sorbed to (clay) mineral surfaces. The observation that sorption increased further when 0.01 $mol/L Ca^{2+}$ was added indicates that the increased Ca-oxytetracycline complexation enhanced sorption enough to allow the complexes to out-compete the extra added Ca^{2+} for exchange sites.

However, at 0.1 mol/L added of $CaCl_2$, the oxytetracycline sorption to the soil sample was strongly suppressed, with the slope of the sorption isotherm (Fig. 1.6) only about 1/3 that of the isotherm at 0.01 mol/L added $CaCl_2$. Presumably, the high Ca^{2+} concentration results in much larger competition for sorption sites, resulting in sorption of fewer Caoxytetracycline complexes.

Figure 1.7 shows oxytetracycline sorption to soil 23 at three different concentrations of added NaCl (zero, 0.01, and 0.1 mol/L) and several oxytetracycline concentrations (ranging from 200 μ g/L to 1000 ug/L). The study would expect that the isotherms described by blue dots in both Figures 1.6 and 1.7 would be the same, since both are for soil 23 with no added salts. The two differed somewhat, with isotherms slopes of 0.060 L/g in Fig.1.6 and 0.044 L/g in Fig. 1.7. In contrast to Fig. 1.6 when increasing the Ca^{2+} concentration by 0.01 m/L increased the oxytetracycline sorption, when the Na^+ concentration was increased by 0.01 mol/L (Fig. 1.7), the sorption of oxytetracycline decreased. This can be rationalized by noting that the amount of Ca^{2+} in the system stays the same in all three isotherms of Fig. 1.7. If we assumed that the Na^+ concentration is to provide more competition by Na^+ that result in less sorption of Ca^{2+} oxytetracycline complexes. Since Na^+ is a weaker competitor than Ca^{2+} 0.1 mol/L, Na^+ suppressed oxytetracycline sorption less than 0.1 $mol/L Ca^{2+}$ did.



Figure 1.6 The oxytetracycline sorption to soil 23 at three different concentrations of $CaCl_2$.

Figure 1.8 shows oxytetracycline sorption to soil 4 at three different concentrations of added $CaCl_2$ (zero, 0.01, and 0.1 mol/L) and several oxytetracycline concentrations (ranging from 200 μ g/L to 1000 μ g/L). The results show a significant oxytetracycline sorption to soil 4 at zero added $CaCl_2$ (pure water), but the isotherm slope was 0.023 L/g (Fig. 1.8), about half the slope observed for soil 23 (Fig. 1.6). However, at 0.01 mol/L added of $CaCl_2$, the oxytetracycline sorption to the soil sample decreased slightly while the sorption of oxytetracycline decreased significantly at 0.1 mol/L added of $CaCl_2$.

Figure 1.9 shows oxytetracycline sorption to soil 4 at three different concentrations of added NaCl (zero, 0.01, and 0.1 mol/L) and several oxytetracycline concentrations (ranging



Figure 1.7 The oxytetracycline sorption to the soil 23 at three different concentrations of NaCl.

from 200 μ g/L to 1000 μ g/L). The oxytetracycline sorption to soil 4 has an isotherm slope of about 0.005 L/g for all three conditions, zero, 0.01, and 0.1 mol/L added NaCl. Again, Figure 1.10 shows oxytetracycline sorption to soil 4 with no added salt and at NaCl or $CaCl_2$ concentrations of 0.1 mol/L, and it included several oxytetracycline concentrations (ranging from 200 μ g/L to 1000 μ g/L). The results with no added salt show an isotherm slope of 0.006 L/g, which compares with slopes at the same conditions of 0.023 L/g (Fig. 1.8) and 0.005 L/g (Fig. 1.9). Despite this variability, the trends agree in that the high



Figure 1.8 The oxytetracycline sorption to soil 4 at three different concentrations of $CaCl_2$.

 Ca^{2+} concentration suppresses sorption to soil 4, while high Na^+ concentration has little effects on oxytetracycline sorption.

To summarize oxytetracycline sorption to soils 23 and 4, it seems clear that Ca^{2+} concentrations of 2 to perhaps 15 $mmol/L Ca^{2+}$ contribute very favorably to oxytetracycline sorption to these soils at pH 7.5. This supports the hypotheses that Ca-oxytetracycline complexes are important species that control sorption to clay minerals, Parolo et al. and Aristilde et al. [7,31]. Larger Ca^{2+} concentrations approaching 100 mmol/L strongly suppressed oxytetracycline sorption presumably through competition for cation exchange sites. Na^+ concentrations, on the other hand have little effect on oxytetracycline sorption.



Figure 1.9 The oxytetracycline sorption to the soil 4 at three different concentrations of NaCl.

1.3.2 Sorption at Higher Concentrations

Since all the isotherms above are basically linear, the study used some higher oxytetracycline concentrations to test whether a sorption maximum could be observed. Figure 1.11 shows oxytetracycline sorption to soil 19 without adding additional NaCl or $CaCl_2$ and initial oxytetracycline concentrations ranging up to 5000 μ g/L. The soil mass was about 500 mg. The results show a significant oxytetracycline sorption to soil 19 without reaching maximum.



Figure 1.10 Oxytetracycline sorption to soil 4 at three different concentrations of $CaCl_2$ or NaCl.

In fact, sorption accelerates as oxytetracycline concentrations increase, with the isotherm slope increasing from about 0.01 L/g (up to about 1000 µg oxytetracycline/L) to about 0.034 L/g at higher concentrations. These slopes are larger than most of those for soil 4 but smaller for those for soil 23.

In order to double check the curvilinear nature of Figure 1.11, the study repeated this isotherm while reducing the amount of soil in each batch from 500 mg to 200 mg. Figure 1.12 shows again the oxytetracycline sorption to soil 19 was significant without reaching



Figure 1.11 Oxytetracycline sorption to soil 19 when soil mass about 0.5 g with no added $CaCl_2$ and NaCl.

any maximum. The data in Fig. 1.12 affirm that the isotherm slope increases as sorption increases over the observed range.

Figure 1.13 combines and compares the sorption data of Fig. 1.11 and Fig. 1.12, showing oxytetracycline sorption to soil 19 when soil mass was about 500 mg versus 200 mg. The results show that the sorption of OTC was significantly higher when the soil mass was 200 mg. This might be interpreted in terms of the accelerating (S-shaped) isotherms, for which sorption is increasingly favorable as sorption increases. That is, when a smaller amount of soil is suspended in oxytetracycline solution, much more sorption per unit soil mass must occur in order to reach a given aqueous phase oxytetracycline concentration. At a given aqueous-phase concentration then, the larger sorbed mass on the smaller quantity of soil



Figure 1.12 Oxytetracycline sorption to soil 19 when soil mass about 0.2 g with no added $CaCl_2$ and NaCl.

seems to induce a larger equilibrium quantity of sorbed tetracycline compared to the system containing a larger quantity of soil. Minerals like those found in these Saudi soils could be used to remove tetracyclines from polluted water systems. A number of other palygorskite rich materials have been already proposed for removing tetracycline from contaminated waste streams [33, 46, 47].

The data of the current study showed that oxytetracycline (OTC) sorption was strongest for soil 23 even though soil 23 has the lowest clay and organic carbon contents. In terms of the data that the study gathered, soil 23 had the largest water soluble Ca^{2+} concentration (Table 1.3). This support the hypotheses that higher calcium content contributes to oxytetracycline sorption through complexation. In the other hand, the precise mineralogies remain unknown



Figure 1.13 Oxytetracycline sorption to soil 19 at two different soil masses (0.5 g and 0.2 g). and it may be the soil 23 contains a mineral component that has a greater affinity for oxytetracycline.

1.3.3 Sorption Coefficient (K_d)

For comparison with other data from the literature, sorption coefficient (K_d) values and sorption per unit mass of soil organic carbon (K_{oc}) values for each soil sample were calculated (Table 1.4). The results show that soil 23 has highest K_d and K_{oc} values; 60 and 23000 L/kgSOC, respectively.

Previously measured/estimated K_d values for oxytetracyclines were ranging from 420 to 1030 L/kg as reviewed by Kumar et al. [48]. For a smectite containing Drummer soil from Indiana at pH 7.5 the K_d for three tetracyclines averaged about 2500 L/kg [24]. Another review of tetracycline sorption, in the pH range from 5 to 8.5, to a wide variety of soils [49]

Sample #	$Ca^{2+}(meq/L)$	$Na^+(meq/L)$	Clay $(\%)$	$K_{oc} \ (L/kg)$	$K_d \ (L/kg)$
4	84	152	60	3800	23
19	73	147	32	5000^{*}	23^{*}
23	190	189	18	23000	60

Table 1.4 Sorption coefficient (K_d) and sorption per unit mass of soil organic carbon (K_{oc}) values for plain soils; and comparison between the three selected soils.

* These values were derived from averaging over the entire the data set, even though the slope was not uniform across all the data (Figure 1.13).

found K_d values extending from 40 to 10000 L/kg. This means that the largest K_d value found in this research (60 L/kg) barely overlaps with the range of previous observation of soils.

The Saudi Arabian soils used in this study were relatively poor in organic matter which may have reduced sorption compare to other soils. However, the combination of relatively high clay content and divalent cations such as Ca^{2+} should enable tetracycline sorption. For example, a K_d of about 60000 L/kg was recently observed [32] for sorption of tetracycline to montmorillonite at pH 7.7. This means that a soil containing only 1% montmorillonite could have K_d of 600 L/kg. From the literature, the study found that Al-Ahsa city, which is in eastern region of Saudi Arabia, topsoils are rich in palygorskite and smectite as substantial clay content [1,34]. Both of these minerals could contribute to tetracyclines sorption.

1.4 Conclusions from Experimental Work

There is a tendency to think that sorption of the tetracycline antibiotics to soils increases with decreasing pH, because the cationic forms are more strongly sorbed [21–23]. This tendency would imply minimal tetracycline sorption to desert soils. The hypothesis of this study, based on the work of Parolo et al. and Aristilde et al., is that sorption of tetracyclines to desert soils

could be significant at pH 7.5 because of sorption of Ca-oxytetracycline cationic complexes to clay mineral surfaces. This hypothesis was supported by the data from the current study at low concentrations because all three clayey soils at pH 7.5 sorbed significant quantities of oxytetracycline and Ca-soils sorb more OTC than Na-soils. However, these desert soils have concentrations in excess of 0.1 mol/L (Table 1.3), and at these high concentrations it looks like Ca^{2+} competition suppresses OTC sorption more than Na^+ . However, the soils still have substantial affinities for tetracyclines. While supporting the major hypotheses, these data raise many questions about mineralogy, mechanism, and other geochemical controls which may need further investigations.

Chapter 2

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

2.1 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 [50]. In 2010, the usage of antimicrobials in animal foods reached approximately 13.5 million kg [51, 52].

As mentioned earlier, the tetracycline is a broad-spectrum antibiotic that was discovered in early of 1950s [53]. The chemical structure of tetracycline has explained and showed in Chapter 1 and Figure 1.1. In the aqueous phase, the functional groups in a tetracycline structure often become ionized and cause tetracycline to form cation, zwitterion (neutral), or anion species, depending on solution pH. It is showed in Chapter 1 and 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 of tetracycline in 2010 [52, 54].

Since large fractions of tetracycline are not metabolized in the animals, significant amounts of tetracycline are excreted with animal manures, either as the original compound or as its bioactive metabolites [52, 55, 56]. Thus, tetracycline and its derivatives are transferred to soil [57, 58], surface water [59–62], and groundwater [58, 63, 64].

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. Also, it is showed in Chapter 1 and 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 1.3in Chapter 1). 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 [65] 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 [66], 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), [67] 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 was to develop a quantitative model for tetracycline speciation in even more complex systems like soils. This current study builds upon previous work [3] by developing new thermodynamic terms and modeling additional data to create a more comprehensive Phreeqc model of the system. A main goal was 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 in particular have been presented and used by Parolo et al. [7] and Aristilde et al. [31] and incorporated in this work to help fit Phreeqc results 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 2.1. Those equations and parameters that were newly developed for this study are highlighted in bold. In Werner et al. [4], they measured tetracycline speciation and complexation by Ca^{2+} , and Mg^{2+} as
functions of pH. The study selected their parameters 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 [5]. In using 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 2.1 for cation I^{i+} are referenced to this equation [5]:

$$Na^+ + 1/i \cdot I - X_i \iff Na - X + 1/i \cdot I^{i+}$$

$$(2.1)$$

The Phreeqc database compiles all such reactions as half-reactions of the form [5]:

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

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 halfreaction. Since logk = 0, K_{exch} must equal 1, and so if any other cation-exchange halfreaction 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^+ [5].

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 2.1) is:

$$K^+ + X^- = KX \quad logk = 0.7$$
 (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 2.1) is:

$$Ca^{2+} + 2X^{-} = CaX_2 \quad logk = +0.8$$
 (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 $[\triangle G = -RT lnk = -RT(2.3) logk]$ 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:

$$K^{+} + 1/2Ca - X_2 \longrightarrow KX + 1/2Ca^{2+} \quad logk = +0.3$$
 (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 that potassium is less competitive than calcium at low ionic strength, and as ionic strength increases the potassium becomes more competitive with calcium. And then these parameters have been used in many modeling studies of cation exchange involving K^+ and Ca^{2+} [68–70].



Figure 2.1 Calculated equilibria for K- Ca-exchange using Phreeqc and the cation exchange coefficients of Appelo and Postma [5] and Table 2.1 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

The work of Yunjie Ding [6] measured the cation exchange equilibria of tetracycline with Kand 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 [6] 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 (Figure 1.2). 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

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

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

could be inferred. Third, note that Figure 1.3 shows that the $CaH_2(Tec)^+$ complex should be an important tetracycline species in calcium systems [4]. Sorption of that complex should result in one Ca^{2+} consumed from solution along with each two tetracycline sorbed, by the reaction:

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

$$H_4(Tec)^+_{(aq)} \iff H_3(Tec)^o_{(aq)} + H^+_{(aq)} \iff H_2(Tec)^-_{(aq)} + 2H^+_{(aq)}$$
(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 [6] 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:

$$H_4 TecCl \longrightarrow H_4 Tec^+ + Cl^- \tag{2.13}$$

$$H_4 Tec^+ \longrightarrow H^+ + H_3 Tec^0 \tag{2.14}$$

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 [71], 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/kgwof $NaHCO_3$ 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 [6], but in order to fit pH properly, this buffer was used in all subsequent calculations.



Figure 2.2 Comparison of modeled and experimental [6] pH values of initial tetracycline solution.

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 2.1.

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 [6], 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 [6]. A plausible reason for this discrepancy is explained by Ding p.88 of [6]:

"The basal spacing of smectites were 14.7 Å with tetracycline loadings $< 135 \ \mu mol/g$... the distance between two adjacent clay sheets was 5.1 Å ... 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 Å. 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 [6] 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 [72], 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) [73], and so many smectites should display plateaus or inflections in their tetracycline sorption near $42 - 43 \ cmol(+)/kg$. For example, Figueroa et al. [27] studied sorption of oxytetracycline and tetracycline to Namontmorillonite. 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., [74]) also commented that the sorption results of Figueroa et al. [27] seem low by a factor of about 10. Furthermore, the same authors (Li et al., [74]) 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. [75] 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 a dilute solution (no added salt other than tetracycline HCl and the carbonate buffer discussed above), while the remaining three cases added an additional 0.01 M, 0.1 M, and 0.8 *molal* as KCl. Note that this study uses different units at the highest ionic strength, since molarity and molality begin to significantly diverge at such concentrations.

The study adjusted the new thermodynamic parameters to fit Ding's results [6]. 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 [6] in dilute solution (no added salt other than tetracycline HCl and sodium carbonate) for tetracycline sorption by K-smectite, and the result was fit very well. Note that Essington et al. [76] 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 [6] at 0.01 M 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 [6] at 0.1 M 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 [6] at 0.8 *molal* 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 the K-smectite clay in dilute solution (no added KCl).

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 Ca_2HTec^{2+}/Ca^{2+} and CaH_2Tec^+/Ca^{2+} .

In the aqueous phase where tetracycline and cations such as calcium are present, the tetracycline will form complications with cations depending on solution pH phase (Figure 2.7).

The current study focuses on three sorption parameters: $H_4(Tec)^+$, $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$. Summing these three terms results in total Tec sorption for Ca-systems, in this model. Given that extensive modeling supports the use of log k = 3.8 for sorption of the



Figure 2.4 The sorption of tetracycline to the K-smectite clay at $0.01 \ M \ KCl$ ionic strength.



Figure 2.5 Sorption of the tetracycline to the K-smectite clay at 0.10 $M\ KCl$ as ionic strength.



Figure 2.6 Sorption of tetracycline to the K-smectite clay at 0.80 molal KCl ionic strength.



Figure 2.7 Ca^{2+} and tetracycline speciation and complication in aqueous phase as a function of pH solution which calculated using Phreeqc model [7]. These calculations were done with 0.00076 mol/L tetracycline in calcium chloride with ionic strength of 0.004 M.

 $H_4(Tec)^+$ species, this study explored the parameter space offered by the $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ terms. When the $Ca_2H(Tec)^{2+}$ term was commented out in the database

(meaning that $Ca_2H(Tec)^{2+}$ sorption was not allowed in Phreeqc), the log k for $CaH_2(Tec)^+$ was varied across values from 3 to 7. With a log k for $CaH_2(Tec)^+$ of 7, Phreeqc predicted tetracycline sorption $(H_4(Tec)^+ \text{ plus } CaH_2(Tec)^+)$ that fit the experimental data very well (Figures 2.8 and 2.9). At higher ionic strengths, the Phreeqc predictions were increasingly too large compared to experiment (results not shown), implying that any larger values of $\log k$ for $CaH_2(Tec)^+$ were unrealistic at high ionic strengths, even though results at low ionic strengths were promising.



Figure 2.8 Sorption of tetracycline to Ca-smectite in dilute solution (no added salt other than tetracycline HCl and sodium carbonate) when $\log k$ value for $CaH_2(Tec)^+$ was set to 7.



Figure 2.9 Sorption of tetracycline to Ca-smectite at 0.01 M ionic strength when log k value for $CaH_2(Tec)^+$ was set to 7.

This study's other significant Ca-related variable is the log k for sorption of $Ca_2H(Tec)^{2+}$ complexes by smectites, and so this variable was explored while commenting out the $CaH_2(Tec)^+$ term. Again, with log k = 3.8 for sorption of the $H_4(Tec)^+$ species, the log k for sorption of $Ca_2H(Tec)^{2+}$ was varied from 3 to 6.6. With a log k for $Ca_2H(Tec)^{2+}$ of 6.6, Phreeqc predicted tetracycline sorption ($H_4(Tec)^+$ plus $Ca_2H(Tec)^{2+}$) that followed the experimental trends fairly well (Figures 2.10 and 2.11). At higher ionic strengths, the Phreeqc predictions were much too large compared to experiment (results not shown), implying that any larger values of log k for $Ca_2H(Tec)^{2+}$ were unrealistic at high ionic strengths, even though results at low ionic strengths were promising.



Figure 2.10 Sorption of tetracycline to Ca-smectite in dilute solution (no added salt other than tetracycline HCl and sodium carbonate) when $\log k$ value for $Ca_2H(Tec)^{2+}$ was set to 6.6.

The next question for this study, then, was to characterize the combined behavior of the parameters for $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ sorption. Somewhat surprisingly, both parameters needed to be maintained near their individual maxima. That is, if log k values for both terms were set to 4, then sorption was greatly underpredicted under conditions of medium-to-high pH and low ionic strengths (Figure 2.12).

A better fit of the sorption data was obtained when the parameters for $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ sorption were each set to 6.6 (Table 2.1). Figure 2.13 shows the comparison between these Phreeqc predictions and Ding's data [6] in dilute solution (no added salt other than tetracycline HCl and sodium carbonate) for tetracycline sorption by Ca-smectite. The result was fit well, but the prediction of Phreeqc was a little bit lower than Ding's data.



Figure 2.11 Sorption of tetracycline to Ca-smectite at 0.01 M ionic strength when log k value for $Ca_2H(Tec)^{2+}$ was set to 6.6.

Figure 2.14 compares the Phreeqc predictions for tetracycline sorption by Ca-smectite with Ding's data [6] at an ionic strength of 0.01 M as $CaCl_2$. Also, the agreement between model and experiment was fit well, but the model predictions were again a little bit lower than the experimental data. This parameter set does a decent job of predicting tetracycline sorption at low ionic strengths. However, just as with the individual $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ parameters, when both were set to 6.6, the Phreeqc predictions for tetracycline sorption at high-Ca ionic strengths of 0.10 and 0.82 molal were much too large compared with experiment (see below).



Figure 2.12 Sorption of tetracycline to Ca-smectite in dilute solution (no added salt other than tetracycline HCl and sodium carbonate) when $\log k$ values for both $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ species were set to 4.

Figure 2.15 shows the comparison between the Phreeqc predictions and Ding's data [6] at an ionic strength of 0.10 M CaCl₂ when the log k values for $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ sorption were each set to 6.6 (Table 2.1). The Phreeqc prediction of tetracycline sorption followed the experimental trend, but the predictions from Phreeqc were higher than Ding's data. Nevertheless, the study did many attempts to vary these two log k values, and followed several different approaches to reach better Phreeqc predictions of the experimental data. Given that the log k for $H_4(Tec)^+$ sorption is set to 3.8, if the 0.1-M sorption data are to be fit, then the current research found that the log k values of both $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ species should be reduced from 6.6 to 5.6. Therefore, Phreeqc was capable of predicting the experimental data of Ding when $CaCl_2$ was at 0.10 M, but the fit required different parameters than at lower ionic strengths. Under these conditions, Figure 2.16 shows



Figure 2.13 Sorption of tetracycline to Ca-smectite clay in dilute solution (no added salt other than tetracycline HCl and sodium carbonate).

very good Phreeqc predictions of tetracycline sorption, and the Phreeqc results were very close to Ding's observation data.

Figure 2.17 shows the comparison between the Phreeqc predictions and Ding's data [6] at an ionic strength of 0.82 molal $CaCl_2$ when the log k values for $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ sorption were each set to 6.6 (Table 2.1). The Phreeqc sorption predictions were far higher than Ding's data, illustrating that many Ca-tetracycline complexes form and sorb when calcium is plentiful. The study again did many attempts to vary the $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ log k values, and followed several different approaches to reach better Phreeqc predictions of the experimental data. Again, fixing the log k for $H_4(Tec)^+$ sorption to 3.8, if the 0.82-molal sorption data are to be fit, then the current research found that the log k values of both $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ species should be reduced from 6.6 to 4.4. Under those conditions, Phreeqc was again capable of predicting the experimen-



Figure 2.14 Sorption of the tetracycline to Ca-smectite clay at 0.01 $M \ CaCl_2$ ionic strength.

tal data (Ding) gathered at 0.8 M CaCl₂, but the fit again required different parameters than at lower ionic strengths. Then, Figure 2.18 shows very good Phreeqc predictions of tetracycline sorption, and the Phreeqc results were very close to Ding's observation data. A truly self-consistent model would require only one thermodynamic equilibrium constant for each reaction. The need for the present study to change the log k values for sorption of Ca-tetracycline complexes at high ionic strengths indicates that something important (important reactions may be neglected, activities may not modeled well, etc) is still.



Figure 2.15 Sorption of tetracycline to Ca-smectite at 0.10 M ionic strength strength when the log k values of both $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ species were set to 6.6.

Phreeqc allows cation exchange sorption to Ca-clay minerals for three species of tetracycline, $H_4(Tec)^+$, $CaH_2(Tec)^+$, and $Ca_2H(Tec)^{2+}$ species. Formation of each of these species depends strongly on pH, and so their contributions to overall tetracycline sorption also depend on pH. In the solid phase at an ionic strength of 0.01 M, Figure 2.19 shows the Phreeqc calculated contributions of these three sorbed tetracycline species to the total sorption. Note that above pH of 7 the $Ca_2H(Tec)^{2+}$ species dominate sorption, indicating the importance of this species for tetracycline sorption in desert soil environments. Aristilde et al. [31], show the importance of tetracycline complexation with Ca^{2+} or Mg^{2+} for tetracycline sorption to clay minerals at high pH, and Parolo et al. [7] emphasize the importance of this $Ca_2H(Tec)^{2+}$ species when calcium-tetracycline ratio is large. Werner et al. [4]



Figure 2.16 Sorption of tetracycline to Ca-smectite at 0.10 M ionic strength strength when the log k values of both $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ species were set to 5.6.

argued for the dominance of $CaH_2(Tec)^+$ species near neutral pH. Phreeqc used Werner et al. complexation constants but our Phreeqc modeling for sorption of these complexes to clay minerals indicates that $CaH_2(Tec)^+$ species dominates tetracycline sorption all the way down to pH of 4.5 (Figure 2.19). Sorption of tetracycline near pH of 4 is relatively independent of calcium [31], which implies that the $H_4(Tec)^+$ species dominates sorption. Figure 2.19 also shows that sorbed concentration of $H_4(Tec)^+$ and $CaH_2(Tec)^+$ species are comparable even at the lowest pH, implying that sorption processes in soil drive tetracycline into the sorbed $CaH_2(Tec)^+$ species at pH values well below those at which this species would be prevalent in solution phase if the soil CEC were not present (Figure 2.7).

Then, the current results make it tempting to believe that a good overall fit has been found using the modeling parameters compiled in Table 2.1. Thus, great care must be advised when



Figure 2.17 Sorption of tetracycline to Ca-smectite at 0.82 molal ionic strength when the $\log k$ values of both $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ species were set to 6.6.



Figure 2.18 Sorption of tetracycline to Ca-smectite at 0.82 molal ionic strength when the $\log k$ values of both $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ species were set to 4.4.



Figure 2.19 Contributions of three tetracycline species to the total sorption calculated using Phreeqc model at an ionic strength of 0.01 M.

using goodness-of-fit as the main criterion for selecting thermodynamic modeling parameters.

2.3.2 Inorganic Cations Released When Tetracyclines Sorbed to Clay

Ding measured the release of inorganic cations from clay for his systems where tetracycline sorbed in the absence of added salt [6]. The smallest nonzero ionic strength the Ding used was 0.01 M while the maximum observed inorganic cation release was 0.0002 M under his experimental conditions, and so cation release can be only measured for the case of dilute solution (no added salt other than tetracycline HCl). This study used Phreeqc to predict K^+ and Ca^{2+} released to the solution phase from the smectite clays in dilute solution (no added salt other than tetracycline HCl and sodium carbonate). Figure 2.20 shows comparisons between Phreeqc predictions and Ding's experimental data for both (a) the amount of tetracycline sorbed to K-smectite clay and (b) the amount of K^+ released to the solution. The experimental results show that the amount of K^+ released was increased when tetracycline concentration was increased, and the K^+ released was always less than or equal to the tetracycline sorbed. The Phreeqc results show that K^+ released was greater than tetracycline sorption, especially at low concentrations. This is an artifact of using the sodium carbonate buffer (Figure 2.2) to match the experimental pH values. The 0.12 $mmol/L Na^+$ concentration in that buffer removed K^+ from the clay by cation exchange. Perhaps potassium carbonate would have been a better choice for buffering the pH, because would have allowed this study to measure K^+ released without competition from Na^+ .



Figure 2.20 Tetracycline sorption to K-smectite clay and K^+ released calculated using Phreeqc in dilute solution (no added salt other than tetracycline HCl and sodium carbonate).

Figure 2.21 shows comparisons between Phreeqc predictions and Ding's experimental data [6] for both (a) the amount of tetracycline sorbed to Ca-smectite clay and (b) the amount of Ca^{2+} released to the solution. But here, the Phreeqc results show that the

amount of Ca^{2+} released to the solution was very low compared to the experimental results, for all amounts of tetracyclines sorbed to the clay. This implies that too much Ca^{2+} is retained in the clay when tetracycline sorbed, so probably the log k for sorption of Catetracycline complexes is too large. When log k for the sorption of $CaH_2(Tec)^+$ species was reduced from 6.6 to 5.6, then the log k for sorption of the $Ca_2H(Tec)^{2+}$ species had to be increased from 6.6 to 10.2 to get reasonable fit of Ding's experimental data as it shown in Figure 2.22. Overall, the fit of the tetracycline sorption data was much better when both these log k values were 6.6 as it shown in Figure 2.21, even though the calcium release data were not fit well.



Figure 2.21 Tetracycline sorption to Ca-smectite clay and Ca^{2+} released calculated using Phreeqc in dilute solution (no added salt other than tetracycline HCl and sodium carbonate) when log k values of both $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ species were set to 6.6.



Figure 2.22 Tetracycline sorption to Ca-smectite clay and Ca^{2+} released calculated using Phreeqc in dilute solution (no added salt other than tetracycline HCl and sodium carbonate) when log k for $CaH_2(Tec)^+$ species was set to 5.6 while log k for $Ca_2H(Tec)^{2+}$ species was set to 10.2.

2.3.3 Fraction of Tetracycline Apparently Sorbed as Uncomplexed Cations

This study also used Phreeqc to calculate the percentage of tetracycline apparently sorbed as uncomplexed cations. In Chapter three of Ding's dissertation [6], Figure III-2 showed inorganic cations exchanged/released as a function of tetracycline sorption. This modeling study calculated the relevant species and then compared these species to estimates from Ding's data. For K-smectite, the study assumed one $H_4(Tec)^+$ molecule sorbed for each potassium cation exchanged/released. For Ca-smectite, the study assumed that two $H_4(Tec)^+$ molecules needed to sorb in order to release one Ca^{2+} to solution as estimated from Ding's experimental data [6]. Figure 2.23 shows comparisons between tetracycline percentages sorbed to potassium smectite clay as cations in Phreeqc versus the estimated percentages of tetracyclines sorbed as cations from Ding's experimental data. The results show that the Phreeqc prediction of proportions of tetracycline sorbed as cations were very close to the estimated percentages for tetracycline cation sorption from Ding's experimental data. Moreover, the results show that the percentages of tetracyclines apparently sorbed as cations increased when tetracycline concentration was increased in both Ding's experimental data and Phreeqc prediction results.



Figure 2.23 Percentage of tetracyclines sorbed as cations to K-smectite clay in dilute solution (no added salt other than tetracycline HCl and sodium carbonate).

Figure 2.24 shows comparisons between tetracycline percentages sorbed to calcium smectite clay as cations in Phreeqc versus the estimated percentages of tetracyclines sorbed as cations from Ding's experimental data.

The results show that the Phreeqc prediction of proportions of tetracycline sorbed as

cations were fairly close to the estimated percentages for tetracycline cation sorption from Ding's experimental data. Again, the results show that the percentages of tetracyclines apparently sorbed as cations increased when tetracycline concentration was increased in both Ding's experimental data and Phreeqc prediction results.

For calcium, there was greater discrepancy between modeling and experimental results than the case of potassium. As noted above, Phreeqc under predicted the amount of calcium released when tetracycline sorbed, but apparently the relative proportions of calciumcomplexed versus uncomplexed tetracycline cations were more accurate.



Figure 2.24 Percentage of tetracyclines sorbed as cations to Ca-smectite clay in dilute solution (no added salt other than tetracycline HCl and sodium carbonate).

Section in Phreeqc	Reactions	log_k
Database		
SOLUTION_MASTER	Tec $H_4(Tec)^+$	b
SPECIES		
SOLUTION_SPECIES	$H_4(Tec)^+ = H_4(Tec)^+$	0.000^{d}
	$H_4(Tec)^+ = H_3(Tec) + H^+$	-3.45^{a}
	$H_4(Tec)^+ = H_2(Tec)^- + 2H^+$	-11.45^{a}
	$H_4(Tec)^+ = H(Tec)^{2-} + 3H^+$	-21.23^{a}
	$H_4(Tec)^+ = Tec^{3-} + 4H^+$	-33.64^{a}
	$\mathbf{Ca^{2+}} + \mathbf{H_2}(\mathbf{Tec})^- = \mathbf{CaH2}(\mathbf{Tec})^+$	3.4^b
	$Mg^{2+} + H_2(Tec)^- = MgH_2(Tec)^+$	3.9^{b}
	$\mathbf{Ca^{2+}} + \mathbf{H}(\mathbf{Tec})^{2} - = \mathbf{CaH}(\mathbf{Tec})$	5.8^{b}
	$Mg^{2+} + H(Tec)^{2-} = MgH(Tec)$	4.1^{b}
	$\mathbf{Ca^{2+}} + \mathbf{H_3}(\mathbf{Tec}) = \mathbf{CaH_3}(\mathbf{Tec})^{2+}$	3.0^d
	$K^+ + H_2(Tec)^- = KH_2(Tec)$	1.04^{e}
	$\mathbf{K^+} + \mathbf{H_3}(\mathbf{Tec}) = \mathbf{KH_3}(\mathbf{Tec})^+$	1.04^{e}
	$\mathbf{2Ca^{2+}+H(Tec)^{2-}=Ca_2H(Tec)^{2+}}$	8.671^{g}
PHASES	$\operatorname{Fix}_{-}H^{+}$	0.00^{d}
	$H^+ = H^+$	
EXCHANGE_MASTER	$Y = Y^{-}$	$__d$
SPECIES		

Table 2.1 Compilation of all chemical equations and equilibrium constants used in the present study. All equations are written as they appear in the Phreeqc model database.

Table 2.1 (cont'd)

Section in Phreeqc	Reactions	log_k
Database		Ŭ
EXCHANGE_SPECIES	$X^- = X^-$	0.0^{f}
	$\mathbf{H_4(Tec)^+} + \mathbf{X^-} = \mathbf{H_4(Tec)X}$	3.8^d
	$\mathbf{CaH_2}(\mathbf{Tec})^+ + \mathbf{X}^- = \mathbf{CaH_2}(\mathbf{Tec})\mathbf{X}$	6.6^d
	$\#\mathbf{CaH_3}(\mathbf{Tec})^{2+} + \mathbf{2X^-} = \mathbf{CaH_3}(\mathbf{Tec})\mathbf{X_2}$	$#4.8^{d}$
	$\mathbf{Ca_2H(Tec)^{2+}+2X^{-}=Ca_2H(Tec)X_2}$	6.6^d
	$MgH_2(Tec)^+ + X^- = MgH_2(Tec)X$	2.0^d
	$\mathbf{KH_3}(\mathbf{Tec})^+ + \mathbf{X}^- = \mathbf{KH_3}(\mathbf{Tec})\mathbf{X}$	3.6^{d}
	$Na^+ + X^- = NaX$	0.0^{f}
	$K^+ + X^- = \mathrm{KX}$	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 from Parolo et al. 2013.

2.4 Conclusions from Modeling of Tetracycline Sorption to K- and Ca-Smectites

The Phreeqc modeling software was self-consistently used to model $K^+/H_4(Tec)^+$ and $Ca^{2+}/H_4(Tec)^+$ exchanges simultaneously, in that one logk value (3.8) for sorption of the $H_4(Tec)^+$ species was shared in systems with background electrolytes of either KCl or $CaCl_2$. The Phreeqc approach was able to model $K^+/H_4(Tec)^+$ exchange fairly well, fitting datasets with KCl ionic strengths from dilute solution (no added salt) to 0.8 M. Fitting these data required addition of one term for clay sorption of $KH_3(Tec)^+$ complexes, using literature values for formation of the complexes in aqueous solution. Modelling the Ca^{2+} -tetracycline system required cation-exchange parameters for both $CaH_2(Tec)^+$ and $Ca_2H(Tec)^{2+}$ species; these parameters were ionic-strength-dependent, since the same set could not reproduce tetracycline sorption results over the entire range of $CaCl_2$ ionic strength. Near pH 7 and above, the Phreeqc modeling results indicated the importance of $Ca_2H(Tec)^{2+}$ species for tetracycline sorption in desert soil environments. While this work developed several thermodynamic relationships for modeling tetracycline sorption to K- and Ca-clays, this study may require further investigations.

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