

ANTIBIOTIC SORPTION AND TRANSPORT IN POROUS MEDIA: EFFECT OF
SOLUTION CHEMISTRY, DIVALENT METALS AND COLLOIDS

By

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To my parents and grandparents

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LIST OF ABBREVIATIONS

| | |
|-------|--|
| CIP | Ciprofloxacin |
| FQ | Flumequine |
| FQs | Fluoroquinolones |
| HPLC | High performance liquid chromatography |
| ICP | Inductively-Coupled Plasma |
| PHzpc | Zero-point-of-charge of metal oxides |
| PKa | Acid dissociation constant |
| PPCPs | Pharmaceuticals and personal-care products |
| SMZ | Sulfamethoxazole |

Abstract of Dissertation Presented to the Graduate School
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Antibiotics have been frequently detected in soils and groundwater; however, their transport behaviors in soils remain largely unknown. The fate and transport of antibiotics in soils are influenced by solution chemistry such as pH, ionic strength, and divalent metals. I examined the effect of solution chemistry on retention and transport of two antibiotics sulfamethoxazole (SMZ) and ciprofloxacin (CIP) under saturated sand media.

Laboratory columns packed with quartz sand were used to test the effects of solution pH and ionic strength (IS). In general, SMZ manifested a much higher mobility than CIP. Almost all SMZ transported through the columns within one pore volume in deionized water, but no CIP was detected in the effluents under the same condition. Perturbations in solution pH and IS showed no effects on SMZ transport in the sand columns. When pH was increased to 9.5, however, ~93% of CIP eluted from the sand columns.

I then examined CIP retention and transport under the influence of Cu and Ca cations by either mixing CIP with Cu/Ca in solution or preloading CIP onto sand then

using Cu/Ca solution to mobilize CIP. Though the amount of Fe/Al oxides on native sand surface was limited, it significantly impeded CIP transport, delaying CIP breakthrough curve. In clean sand where Fe/Al oxides were removed, presence of Ca and Cu both significantly promoted CIP transport by reducing the retardation factor R. In native sand, due to CIP's strong complexation ability with Fe/Al, only Cu promoted CIP transport. Compared to clean sand, native sand not only sorbed 10 times more CIP (50 mg kg⁻¹) than clean sand. Though native sand had limited Fe/Al oxides on surface, they were the major sites for CIP sorption via complexation with CIP's carboxyl group. Strong sorption colloids like montmorillonite can facilitate CIP transport.

In short, the chemical property of antibiotics greatly influenced their fate and transport in porous media with SMZ being much more mobile than CIP. Metal oxides on solid phase surface and solution chemistry including pH, IS and cations significantly influenced the fate of CIP in porous media.

CHAPTER 1 ANTIBIOTICS IN THE ENVIRONMENT

In the past decade, there has been growing concern regarding the release of antibiotics in the soil and water system (Vulliet et al. 2011; Lapworth et al. 2012). Antibiotics are widely used in health care and agricultural industries for the treatment and prevention of diseases. High volume of manufacturing and consumption of antibiotics results in their release into the soil and water system inevitably, which may impose a potential risk on the ecosystems (Nygaard et al. 1992; Brown et al. 2006). Significant increases of antibiotic resistance genes have been observed in many circumstances globally, which is plausibly related to the widespread antibiotics in the environment.

Origin of Antibiotic in the Environment

In the USA, more than 25 million pounds of were manufactured in 2000; more than half of these antibiotics were used for living stock (Threats 2009) . European Federation of Animal health has reported in 1999, more than ten thousand tons of antibiotics has been used in European area, 65%of these antibiotics was in human medicine, 29% was for veterinary usage, and 6% was used for animal for growth promoters ((FEDESA) 1997) . In Europe animal growth promoter usage of antibiotics has already been banned recently; in USA this usage of antibiotics for animal growth still remain. Small amount antibiotics also have been used for plant to control certain bacterial diseases of high-value fruit, vegetable, and ornamental plants. In the USA, typically less than 0.5% of antibiotics applied for plant disease (McManus et al., 2002).

Most of the unused antibiotics have been disposed into the sewage system. Since the sewage system is not designed specifically to remove antibiotics during

sewage treatment so majority of the antibiotics were not treated (Le Corre et al. 2012). These antibiotics will enter the environmental and reach surface water and subsurface water (Lapworth et al. 2012), and even drinking water (Vulliet et al. 2011). Antibiotics used for human or animal treatment or as growth promoters are excreted and end up in manure. Unmetabolized antibiotic substances are often entered the aquatic environment. For example, fluoroquinolone antibiotics, was found in in hospital effluent with concentration as high as $200 \mu\text{g L}^{-1}$, Ampicillin was found in German hospital effluence with concentration between 20 and $80 \mu\text{g L}^{-1}$ (Brown et al. 2006; Martins et al. 2008).

Antibiotics in Soil

There are several environmental factors influencing the toxicity of antibiotics in soils including soil property and chemical characteristics of antibiotics (Kemper 2008) . One of the most important issues for antibiotics in soil is their fate and transport. Antibiotics used for veterinary use are excreted by the animals and often end up in subsurface via manure used as agricultural fertilizer. The loads of antibiotics shed by maturing have been estimated up to kilograms per hectare (Kemper 2008).

Often, antibiotics are released into the subsurface with slight transformation for a relatively long time period (Vulliet et al. 2011). Chemical and physical behavior of these antibiotics in the soil depends on their molecular structure. Antibiotics are classified into ionized, amphiphilic or amphoteric groups, and for this reason, antibiotics can interact with different soil components.

Due to their properties, such as their molecular structure, polarity and solubility, the sorption and desorption of these antibiotics in soils differ significantly (Kümmerer 2001; Carrasquillo et al. 2008) . Some antibiotics like ciprofloxacin (CIP) are charged in

natural pH and slightly water soluble and as a result highly immobile in soils. However, these highly retarded antibiotics can still be mobilized in certain circumstance. For example, the transport of tetracyclines seems to be restricted to fast preferential and macropore flow or to be facilitated by co-transport with mobile colloids such as dissolved organic matter. Most antibiotics are sorbed to solid phase rapidly. Their antibiotic activity is decreased by sorption, however, that does not mean a complete elimination of their antimicrobial activity (Chander et al. 2005). Experimental studies on the antibacterial activity of soil-bound tetracycline and tylosin showed that even though these compounds are tightly adsorbed by clay particles, they remain active (Chander et al. 2005), showing antimicrobial effects that may influence the selection of antibiotic resistant bacteria in the terrestrial environment. If the application of contaminated manure in soil exceeds their degradation rate, it will result in their accumulation. In general, these sorbed compounds serve as a reservoir of pollutants and further contaminate groundwater by leaching.

There are several potential pathways for antibiotics to enter the natural water bodies including groundwater aquifers (Jones et al. 2001; Lapworth et al. 2012). Leaching from soils (e.g., agricultural fields and landfills) is one of the most important pathways for antibiotic transmission to water bodies. For example, large volumes of animal wastes (manure and slurry) containing high doses of antibiotics have been commonly applied to agricultural crop fields as a source of organic fertilizer to increase crop yields. Although some antibiotic may be retained by the soils, the rest can be transported to surface and groundwater through surface runoff and infiltration.

Several studies have been conducted to investigate the mechanisms controlling the retention and release of antibiotics in soils (Heberer et al. 2004; Lorphensri et al. 2007) . Comprehensive investigations ranging from large-scale observations in the field to bench-scale studies with batch experiments have advanced the knowledge of environmental fate of antibiotics. Most of the previous studies, however, mainly focused on their sorption, desorption, and degradation processes in soils. Only few studies examined their transport dynamics in porous media. Therefore, additional investigations are necessary to improve current understanding of how flow dynamics affect the transport behavior of antibiotics in porous media.

The decomposition of antibiotics in subsurface is driven by many factors. Photo degradation has been recognized for sulfonamide group and quinolone group antibiotics, but it is not an important effect in subsurface since the light is reduced there. Antibiotic degradation in subsurface is mainly through microbial activity, especially enzymatic reactions, and oxidative decarboxylation, especially in Fe or Mn enriched soils. Biodegradation in soil increases when manure or sludge with high numbers of micro-organisms is added.

Soil serves as a vast reservoir for micro-organisms (Essington 2003). High numbers of bacteria are important in maintaining mineral immobilization and decomposition processes. Antibiotics are mainly affected in two ways: the microbial community can be significantly changed by antibiotic activities; these environmental bacteria can acquire antibiotic resistance genes by exposing with the antibiotics. Some soil micro-organisms have natural tolerance genes for antibiotics. However, most bacteria, antibacterial resistance genes develop only when exposed to antibiotics

(Riesenfeld et al. 2004). For example, soil microorganisms became resistant after the application of tetracycline-contaminated manure. Regarding this provoked antibiotic resistance genes, an overview of the antibiotic resistance genes in soil bacteria is presented by Riesenfeld et al (2004). Furthermore, in some cases, provoked resistance did not happen in soils, but directly in the manure, then spread to the soil by fertilizing. In Europe, the analysis of numerous manure samples revealed high bacteria resistant characters against various antibiotics. After manure fertilization, tetracycline-resistant bacteria increased in soil and groundwater, but declined after the cessation of slurry fertilized soil within eight months. The ability to take up the special genes from the environment occurs quite commonly among soil and water samples, the role of natural transformation in the extraction of environmental bacterial resistance genes. Mobile genetic elements and transferring resistance were detected in microbial organisms of environmental samples and pig manure.

Antibiotics in Water

For the continued exponential growth in human population, the limited supplies of water resources are one of the most important global issues. In recent years, the occurrence and fate of antibiotics in the nature water body have been subjects to many investigations carried out globally (Zorita et al. 2009; Luo et al. 2011; Vulliet et al. 2011). More than 30 antibiotics have been found in waste water treatment plant effluent samples, in surface waters and even ground and drinking water. The main source of the antibiotics and their metabolites or degradation products is animal husbandry and administered drugs (Kemper 2008). These antibiotics reach the aquatic environment by the application of manure or slurry to areas used for agriculture, or by animals excreting directly on the land. Surface run-off, driftage or leaching will facilitate the antibiotics to

transport in deeper layers of soils. The solubility of most of the antibiotics is relatively high, as a result, it can be carried by water flow (Lapworth et al. 2012)

There are several potential pathways for antibiotics to enter the natural water bodies including groundwater aquifers. Leaching from soils (e.g., agricultural fields and landfills) is one of the most important pathways for antibiotic transmission to water bodies (Thiele-Bruhn 2003; Overcash et al. 2005).

example, animal wastes containing high doses of antibiotics have been commonly applied to agricultural crop fields, most of the antibiotics are washed into the soil and eventually can be transported to surface or groundwater (Lapworth et al. 2012). Although some antibiotic may be retained by the soils, the rest can leachate from soil and be released into the subsurface, but depending on soil conditions it may seep into groundwater or spread laterally until it meets a stream or other surface water (Thiele-Bruhn 2003; Overcash et al. 2005).

The influence of antibiotics on the resident bacterial community in marine environment was reviewed by Nygaard et al (1992). Although the concentrations impacting aquatic bacteria are mainly unknown, antibiotics remaining active against bacteria in the soil and water system have been documented. In wastewater treatment plants, antibiotic resist genes have been detected. These antibiotic resist bacteria enter the environment via biosolids application or wastewater irrigation. It has been reported that aquatic species such as algae and daphnids has also been influenced by antibiotics.

Under laboratory experiment systems, most antibiotic compounds are persistent, with only few being partially biodegraded (Li and Zhang 2010). Even those

biodegraded antibiotics could still have some antibiotic effect. Several studies have been conducted to exam the occurrence of antibiotics in nature water body and effluents from wastewater treatment plants (Zorita et al. 2009).

Tetracycline and ciprofloxacin are not usually expected in aquatic environment due to the high sorption capacity of the soil. However, these substances have been detected in low levels in surface water samples and in higher levels in overland flow water globally (Luo et al. 2011; Vulliet et al. 2011). Antibiotic Sulfonamides, macrolides and fluroquinolone are analyzed frequently in US. Tetracycline was not detected because of their strong adsorption to organic matter and low solubility. The presence of tetracycline-resistant bacterial has been reported by Chee-Sanford et al. (2001) in groundwater underlying two swine production facilities. However, the contribution of antibiotic input from agricultural use is minor in the aquatic environment. The major part of antibiotic input is from human administration via hospital effluents or municipal wastewater (Kümmerer, (2001).

Characteristics and Sorption of Selected Antibiotics in Soils

Sulfamethoxazole (SMZ) and ciprofloxacin (CIP) are typical antibiotics extensively used for both human and veterinary animals. They are among the most frequently detected antibiotics in streams and groundwater in the U.S. (Kolpin et al. 2002; Barnes et al. 2004). SMZ is characterized as a low reactive and high mobile antibiotic (Holten Lützhøft et al. 2000), while CIP is known to have a high affinity to soils (Nowara et al. 1997; Thiele-Bruhn et al. 2004). The different characteristics of CIP and SMZ make them good candidates to represent different antibiotics in the environment. The sorption of antibiotics to soils is controlled by several mechanisms, such as cation bridge interaction, hydrophobic effect, electrostatic interaction, surface complexation,

and ionic exchange. Among these mechanisms, electrostatic interactions are commonly observed for most of the antibiotics including SMZ in soils.

Besides electrostatic adsorption, CIP sorption to soils is affected by surface complexation and cation bridge interaction because it is capable of forming strong complexes with multivalent metal ions on grain surfaces (Riley et al. 1993; Turel et al. 1994). The structure has a bidentate complex between metal ions and CIP through the carboxylic group. This character may be important because the abundance of metals in the environment. Divalent metals like Cu can be accumulated to a relatively high level in contaminated soils. For example, Muchuweti et al. (2006) reported that Cu concentrations in surface soil increased to ~ 230 mg kg⁻¹ through land application of fertilizers, sewage sludge, and wastewater irrigation. In particular, Cu has been used as a feed additive to stimulate animal growth. Li et al. (2007) found that Cu concentrations were 6.86-395 mg kg⁻¹ in swine feeds and 57.0-2,017 mg kg⁻¹ in pig feces. In addition to Cu, Ca is one of the most abundant metals in soils. Hence it is possible that CIP may coexist with Cu or Ca in soils. The formation of complexes between divalent cations and quinolones and its impact on CIP sorption to soils have been studied (Pei et al. ; Wallis et al. 1996; Park et al. 2002). However, little is known about the impact of Cu and Ca on CIP fate and transport in the environment. Therefore, it is important to examine their impacts on the transport behavior of CIP in porous media.

Research Objectives

Several studies have been conducted to investigate the mechanisms controlling the retention of antibiotics in soils (Heberer et al. 2004; Lorphensri et al. 2007; Gielen et al. 2009). Recent research including large- bothfield (Heberer et al. 2004; Lorphensri et al. 2007) and bench-scale studies (Tolls 2001; Pedersen et al. 2003; Gao and

Pedersen 2005; Chefetz et al. 2008) have been conducted to better understand the environmental fate of these antibiotics. However, the majority of recent research mainly focus on sorption, desorption, and degradation processes of. Their transport dynamics in porous media has only been examined in few studies (Kay et al. 2005; Wehrhan et al. 2007; Srivastava et al. 2009; Unold et al. 2009; Unold et al. 2009). Therefore, additional investigations are necessary to improve current understanding of how flow dynamics may affect the transport behavior of antibiotics in porous media. For example, laboratory batch sorption experiments showed that the interactions between antibiotics and soil particles strongly relied on the solution chemistry such as pH and ionic strength (IS). To our knowledge, however, limited investigation has been conducted to evaluate the effects of pH and IS on the transport behaviors of antibiotics in porous media.

The overall objective of this study is to investigate the fate and transport of selected antibiotics in saturated porous media.

In Chapter 2, I hypothesized that both flow chemistry and structural functional group of the antibiotics were the key factors controlling the transport behavior of selected antibiotics. To test the hypothesis, column experiments were conducted to compare the transport behavior of the two antibiotics under different pH and ionic strength. The goal of this study was to determine the influence of solution chemistry on the transport of sulfamethoxazole (SMZ) and ciprofloxacin (CIP) in saturated porous media. Our specific objectives were to: 1) compare the retention and transport of SMZ and CIP in saturated porous media; 2) evaluate the effects of pH and IS on SMZ and CIP transport in saturated porous media; 3) evaluate the effects of ionic strength on the SMZ and CIP transport in saturated porous media; and 4) test whether existing solute

transport models can be used to simulate the retention and transport of SMZ and CIP in saturated porous media.

In Chapter 3, I hypothesized that both sand surface metal oxides and the divalent metal cations have potential influence on CIP sorption onto sand. To test the hypothesis, laboratory batch experiments were conducted to compare the behavior of CIP sorption with two sands(clean and native) and two divalent metals. The goal was to determine the influence of divalent metal on CIP sorption mechanism of CIP in solution. Our specific objectives were to: 1) evaluate the effects of sand surface metal oxides on CIP sorption; 2) evaluate the effects of divalent metals Cu and Ca on CIP sorption; and 3) evaluate the contribution of carboxylic and amine functional groups on CIP sorption onto sand.

In Chapter 4, I hypothesized that both minerals on sand surface and the persence of metal cations have influence on CIP tansport in porous media. Column experimant was designed to better understand the chemodynamic behaviors of CIP insunsurface, which is essential for comprehensive assessment of their potential environmental risk. To test the hypothesis, column experiments using saturated quartz sand were conducted to compare the transport behavior of CIP in two sands and in presence of two metals. The specific objectives were to: 1) evaluate the effects of metal oxides on sand surface on CIP transport in porous media, 2) compare the effects of metals Cu and Ca on retention and transport of CIP in porous media;3) compare the effects of metals Cu and Ca on mobilization of CIP presorbed on porous media; and 4) apply solute transport models to simulate the retention and transport of CIP in porous media.

In Chapter 5, I hypothesized that both minerals on sand surface and colloids have influence on CIP transport in porous media. Similar to chapter 4 we tested the influence of colloids on CIP transport.

Chapter 6 provides a general conclusion and emphasizes the major findings of this study, as well as further research avenues.

CHAPTER 2 EFFECTS OF PH AND IONIC STRENGTH ON SULFAMETHOXAZOLE AND CIPROFLOXACIN TRANSPORT IN SATURATED POROUS MEDIA

Introduction

In the past decade, there has been growing concern over the transport of antibiotics in the environment (Jones et al. 2001). Antibiotics are widely used in health care and agricultural industries for the treatment and prevention of human and animal diseases. High volume of manufacturing and consumption of antibiotics inevitably results in their release into the natural water bodies, which may impose a risk on the ecosystems. Dramatic increase of antibiotic resistance genes have already been observed in many circumstances globally (Johnson et al. 2008; Kozak et al. 2009; Szczepanowski et al. 2009).

There are several potential ways for antibiotics to enter the natural water bodies such as groundwater aquifers. Leaching from soils (e.g., agricultural fields and landfills) is one of the most important pathways for antibiotic transmission to water body (Thiele-Bruhn 2003; Overcash et al. 2005). For example, large volumes of animal wastes (manure and slurry), which contain high doses of antibiotics, have been commonly applied to agricultural crop fields as a source of organic fertilizer to increase crop yields. Although some antibiotic may be retained by the soils, the rest can be transported to surface and groundwater through surface runoff and infiltration (Thiele-Bruhn 2003).

Several studies have been conducted to investigate the processes controlling the retention and release of antibiotics in soils (Heberer et al. 2004; Lorphensri et al. 2007; Gielen et al. 2009). Comprehensive investigations ranging from large-scale observations in the field (Heberer et al. 2004; Lorphensri et al. 2007) to bench-scale studies with batch experiments (Tolls 2001; Pedersen et al. 2003; Gao and Pedersen

2005; Chefetz et al. 2008) have advanced the knowledge of environmental fate of antibiotics. Most of the previous studies, however, mainly focused on the sorption, desorption, and degradation processes of antibiotics in soils. Only a few studies have examined their transport dynamics in porous media (Kay et al. 2005; Wehrhan et al. 2007; Srivastava et al. 2009). Therefore, additional investigations are necessary to improve current understanding of how flow dynamics may affect the transport behavior of antibiotics in porous media. For example, laboratory batch sorption experiments showed that the interactions between antibiotics and soil particles strongly relied on the solution chemistry such as pH and ionic strength. To our knowledge, however, no investigation has been conducted to evaluate the effects of pH and ionic strength on the transport of antibiotics in porous media.

SMZ and CIP are two typical antibiotics extensively used for both human and veterinary animals. They are among the most frequently detected antibiotics in streams and groundwater (Kolpin et al. 2002; Barnes et al. 2004). SMZ is characterized as a low reactive and high mobile antibiotic, while CIP is known to have a high affinity to soils (Nowara et al. 1997; Thiele-Bruhn et al. 2004). The different characteristics of CIP and SMZ make them good candidates to represent a range of antibiotics in the environment. The sorption of antibiotics to soils is controlled by several mechanisms, such as cation bridge interaction, hydrophobic effect, electrostatic interaction, and ionic exchange. Among these mechanisms, hydrophobic and electrostatic interactions are commonly observed for most of the antibiotics including SMZ in soils. In addition to electrostatic interactions, CIP sorption to soil is also affected by cation bridge interaction because it is capable of forming strong complexes with multivalent metal ions on grain surfaces

(Riley et al. 1993; Turel et al. 1994). Nowara et al. (1997) studied the sorption of CIP to clay minerals and suggested that cation bridging was the major sorption mechanism.

The overarching goal of this study was to determine the mechanisms governing the transport of SMZ and CIP in water-saturated porous media. It was our hypothesis that flow chemistry as well as the chemical structure of the antibiotics are the key factors controlling their transport in saturated porous media. To test the hypothesis, laboratory experiments were conducted to compare the breakthrough behavior of the two antibiotics in columns packed with quartz sand under different pH and ionic strength conditions. Previous studies have demonstrated that sand columns can be effectively used in a laboratory environment to mimic porous media under different physicochemical conditions (Gao et al. 2004; Bradford et al. 2005; Bradford and Torkzaban 2008). Our specific objectives were to: 1) compare the retention and transport of SMZ and CIP in saturated porous media; 2) evaluate the effects of pH on SMZ and CIP transport in saturated porous media; 3) evaluate the effects of ionic strength on the SMZ and CIP transport in saturated porous media; and 4) test whether existing solute transport models can be used to simulate the retention and transport of SMZ and CIP in saturated porous media.

Materials and Methods

Materials

SMZ (Sulfamethoxazole, ACS 732-46-6) and CIP (Ciprofloxacin, ACS 85721-33-1) were purchased from Applichem (Germany). The chemical structure and basic information of SMZ and CIP are listed in Table 2-1. All other chemicals were analytical reagents supplied by Fisher Scientific. SMZ stock solution was prepared in methanol at a concentration of 100 mg/L, and CIP stock solution was prepared in acetonitrile at a

concentration of 50 mg/L. Both stock solutions were stored at -20°C in dark prior to use. Acetonitrile and methanol were used in this study as a cosolvent because the solubility of the two antibiotics was relatively low in deionized (DI) water. By diluting the stock solutions with DI water, the final SMZ and CIP concentrations used in this study were $200\ \mu\text{g/L}$ (with 0.1% cosolvent) and $50\ \mu\text{g/L}$ (with 0.5% cosolvent), respectively. All the glassware used in this study was acid-washed before use. The ionic strength (IS) and pH of working solutions were adjusted with KBr and NaOH solutions.

Quartz sand (45/30) sieved to a size range of 0.5-0.6 mm was used as the porous medium (Standard Sand & Silica Co., Davenport, FL) in the column experiments. The sand was washed sequentially by tap water and DI water, and then heated in Fisher Isotemp muffle furnace (Fisher Scientific) at 550°C to remove trace organic impurities. Its zeta potential was determined by measuring the electrophoretic mobility of colloidal quartz sand (Johnson et al. 1996). To obtain colloidal sand, a mixture containing 100 g of clean quartz sand and 200 mL DI water was ultrasonicated for 30 minutes. Aliquots of the quartz colloids were then removed and filtered through a $0.45\ \mu\text{m}$ filter. The filtrate was analyzed for electrophoretic mobility (U) with a ZetaPlus (Brookhaven Instrument Co). The Smoluchowski's formula was used to convert electric mobility of the quartz sand into zeta potential ($\zeta=4\pi\eta U/\epsilon$, where η is the viscosity of the medium and ϵ is the dielectric constant), which was $-19.7\ \text{mV}$.

Analysis of Antibiotics

Reversed phase high-performance liquid chromatography (HPLC, Waters 2695, Milford, MA) equipped with a Phenomenex Gemini C18 column (150 mm \times 4.6 mm I.D., 5 μm) at room temperature was used to determine SMZ concentration. The mobile phase consisted of acetonitrile and sodium acetate aqueous solution (0.02 mol/L, pH

adjusted to 4.75 with acetic acid to maximized column separation effect) at a ratio of 25:75. A Waters 2489 ultraviolet detector was used to detect SMZ at a wavelength of 270 nm. The SMZ detection limit of this method was 20 µg/L and the working range was 50–1000 µg/L with linear correlation coefficients (R^2) > 0.99.

The analysis of CIP was carried out using the HPLC equipped with a Nova-Pak C18 column (150 mm × 3.9 mm, Waters Millipore). The mobile phase consisted of acetonitrile and 0.5% phosphoric acid at a ratio of 15:85. A fluorescence detector (Waters 2475) was used to detect CIP with the excitation and emission wavelengths at 278 and 445 nm, respectively. The CIP detection limit was 5 µg/L and the linear range was 50–600 µg/L with correlation coefficients (R^2) > 0.99.

Column Experiments

To avoid air bubble the quartz sand was wet-packed into an acrylic column measuring 2.5 cm in diameter and 10 cm in height according to the procedures reported by Tian et al. (Tian et al. 2010). A small amount of the quartz sand was poured gently into 8 mL of DI water standing at the bottom of the column until the sand surface was 0.5 to 1 cm below the water level. A polypropylene stir-rod was used to stir the sand in the column. Approximately 8 mL DI water was then added to the column and the column were gently tapped several times to remove air bubble and ensure uniformity. This procedure was repeated several times until the column was packed to a height of 10 cm. Approximately 100 g of sand was used to pack one column with a porosity of 0.42 and a pore volume of 20.6 mL.

A peristaltic pump (Masterflex L/S, Cole Parmer Instrument) was used to control the upward flow at a constant specific discharge of 0.2 cm/min. DI water was first pumped through the saturated column for about 2 h to remove impurities followed by

working solutions overnight to stabilize the pore water pH and ionic strength (IS). For each antibiotic, the experiments were conducted with three different working solutions: DI water (pH=5.7, IS=0), high pH (pH=9.5, IS=0), and high IS (pH=5.7, IS=0.1 mM). Once the outflow was stabilized, the breakthrough experiment was then initiated by switching the inflow to antibiotic solution (i.e., SMZ or CIP).

For all experiments, the antibiotics were applied to the column at a 40 min pulse (i.e., 2 pore volumes-PV), and then the column was flushed with antibiotic-free solution for another 80 min or 4 PVs. The inflow concentrations of SMZ and CIP were 200 and 50 µg/L, respectively, which were within the range of their typical concentrations detected in the environment (Duong et al. 2008; Spongberg and Witter 2008; Karcl and Balcloglu 2009; Zorita et al. 2009). Effluent samples were collected from the top of the column with a fraction collector (IS-95 Interval Sampler, Spectrum Chromatography) during sample injection and column flushing to analyze CIP and SMZ concentrations. All breakthrough experiments were performed in duplicate. Because all the duplications in this study had errors less than 1%, average breakthrough concentrations were reported.

Two sets of breakthrough experiments were conducted for CIP transport in the sand column under DI water conditions. After the pulse-injection of CIP, the column was flushed with CIP-free DI water, specific parameters for each experiment were summarized in Table 2-2. At the end of flushing, the column was separated into 10 layers to determine the concentrations of retained CIP as a function of column depth. The sand was excavated under saturated conditions from top to bottom with a spatula in 1 cm increment and each increment was placed into a small vial with 4 mL of H₃PO₄-KH₂PO₄ buffer and acetonitrile (ACN) solution (i.e., 27.2 g KH₂PO₄ + 1.35 ml H₃PO₄ in 1

liter water with 1:1 ACN). The excavated sand was then washed three times with the same amount of extraction solution. This extraction method has been suggested to be the most effective way to extract fluoroquinolone antibiotics from different types of soils including sandy soils (Uslu et al. 2008). It was verified by our extraction experiments, which showed 100% recovery of retained CIP from the sand. CIP concentrations in the solutions were determined with HPLC. The CIP retention was then calculated for each sand section.

Bromide was applied to the column as a conservative tracer for the breakthrough studies. The experimental procedures were the same as those used for SMZ and CIP. An ion chromatograph (ICS-90, Dionex Corporation) was used to determine bromide concentrations.

Modeling Antibiotic Transport in Saturated Porous Media

One dimensional advection-dispersion equation coupled with reaction terms was used to simulate the transport of CIP and SMZ in water-saturated sand columns. I assumed that the interactions between the antibiotics and the sand grains in the column were affected by both reversible-equilibrium and irreversible-kinetic reactions (Toride et al. 1995). The governing equation can be written as:

$$R \frac{\partial C_w}{\partial t} = D \frac{\partial^2 C_w}{\partial z^2} - v \frac{\partial C_w}{\partial z} - k C_w \quad (2-1)$$

where C_w is the concentration (SMZ or CIP) in pore water ($\mu\text{g L}^{-1}$); R is the retardation factor, which reflects the magnitude of equilibrium reactions in the sand column; D is the dispersion coefficient ($\text{cm}^2 \text{min}^{-1}$); z is the coordinate parallel to flow; v is the velocity of pore water (cm min^{-1}); and k is the kinetic reaction rate constant (min^{-1}).

I solved the governing equation of the transport model numerically for a zero initial concentration, a pulse-input boundary condition at the column inlet, and a zero-concentration-gradient boundary condition at the outlet. The model was first applied to the bromide breakthrough data to estimate dispersion coefficient (D). I assumed that the D of antibiotics was the same as that of the bromide tracer in the column. The transports of SMZ or CIP in the columns were quantified by identifying the best-fit values of the R , and k .

Results and Discussion

SMZ and CIP Transport in Water-Saturated Porous Media

The breakthrough curve of SMZ transport in the sand column in DI water (i.e., pH = 5.6 and IS = 0 mM) was similar to that of bromide, the conservative tracer (Figure 2-1). After the application to the sand column, SMZ was detected in the effluents around 1 PV. The breakthrough curve then quickly moved up and reminded at a peak with further SMZ application. The SMZ concentrations decreased quickly to zero when the columns were flushed with SMZ-free solution. Compared to the bromide breakthrough curve in the column, the breakthrough response of SMZ showed some delay, indicating slight retardation of the antibiotic and/or SMZ had more interaction to sand than bromide in the porous media. The normalized peak concentration (C/C_0) of SMZ was close to unity and mass balance calculations showed that almost all SMZ was transported through the sand column. The breakthrough experiment results demonstrated that SMZ was highly mobile in the water-saturated porous media. This could be attributed to the fact that the interaction between SMZ and porous medium were repulsive because both surfaces were negatively charged under experimental conditions. The zeta potential of the quartz sand used in this experiment was -19.7 mV, confirming it was negatively

charged. At pH of 5.6, about half of the SMZ is negatively charged and the other half is neutral ($pK_{a1}=1.6$ and $pK_{a2} = 5.6$, Table 2-1). Our data are consistent with the literature where many studies have observed the high mobility of SMZ in soils (Delgado1 et al. 2005; Stoob et al. 2007).

Simulations of the transport model matched well with the experimental breakthrough data of SMZ with R^2 of 0.99 (Figure 2-1). The best-fit retardation factor R of SMZ in the column was 1.16 (Table 2-2), indicating slight retardation. The best-fit kinetic reaction rate k of SMZ in the column was zero, suggesting no irreversible-kinetic reactions of SMZ in the saturated porous media.

Different from the transport of SMZ, the transport of CIP in the sand column showed no breakthrough response under DI water condition (pH = 5.6, IS = 0 mol/L) (Figure 2-1). After CIP was applied to the column, the column was flushed with DI water for 2 and 6 PVs (Table 2-2). For both cases, no CIP was detected in the effluents, suggesting no detectable CIP in the pore water within the column due to strong depositions on sand surfaces. At pH 5.6, most of the CIP (80%) in the solutions was positively charged ($pK_{a1}=6.2$ and $pK_{a2} = 8.8$, Table 2-1). The retention of CIP in the sand column therefore could be attributed to the electrostatic interactions (mainly columbic attraction) between positively-charged CIP amine groups and negatively-charged quartz sand surfaces and the surface complexation as discussed before (Gu and Karthikeyan 2005; Hari et al. 2005). Analyses of the retained CIP profile in the sand column after 4 PVs of flushing showed that almost all (~100%) the antibiotic was retained in the bottom 1-cm layer of the column (Figure 2-2), confirming the strong interactions between CIP and the sand grains. Additional flushing of 8 PVs of the

column with DI water mobilized a small portion of the retained CIP, with 95% of the CIP being retained in the bottom layer and the other 5% of CIP being found in the second 1-cm layer from the bottom (Figure 2- 2). This result indicated that although the sorption of CIP onto the sand surfaces was very strong, it was controlled at least partially by the reversible equilibrium reaction (Toride et al. 1995).

The strong CIP retention in this study was consistent with the literature examining its sorption behavior in soil systems (Nowara et al. 1997; Gu and Karthikeyan 2005; Carrasquillo et al. 2008; MacKay and Seremet 2008). As discussed above, CIP sorption onto quartz sand in DI water systems was mainly controlled by two mechanisms: columbic attraction between cationic amine groups of CIP and negatively-charged quartz sand surfaces (Hari et al. 2005) , and surface complexation between carboxyl groups of CIP and the trace level surfacial metal oxides (e.g., iron and aluminum oxides) (Gu and Karthikeyan 2005).

Effects of pH on SMZ and CIP Transport

The transport of SMZ at pH 9.5 in the porous media also exhibited high mobility (Figure 2- 3a). This is because the SMZ solution at pH 9.5 was dominated by negatively- charged species (SMZ^- ; Table 2-1). The transport of SMZ in the sand columns was almost identical for pH 9.5 and 5.6. Unlike SMZ, CIP transport at pH 9.5 showed much higher mobility than that of pH 5.6 (Figures 2-3b). Mass balance calculations showed that ~7% of CIP remained in the sand column at pH 9.5. At pH 9.5, about 83.4% of CIP in the solution was negatively-charged species (CIP^- , Table 2-1), which limited its retention in the column. Our results indicated that pH-dependent

speciation of antibiotics play an important role in controlling their retention and transport in porous media.

Simulations of the transport model matched well with the experimental breakthrough data of both SMZ and CIP with $R^2 > 0.99$ (Figures 2-3a and 2-3b). Because there was no obvious difference in breakthrough curves of SMZ between pH 5.6 and 9.5, the same parameters were used for both conditions. The best-fit retardation factor R of CIP in the column at pH 9.5 was 1.37, which was higher than that of SMZ at 1.16 (Table 2-2). The best-fit kinetic reaction rate k of CIP was > 0 (i.e., 0.007), suggesting part of the CIP might be retained in the sand column through irreversible-kinetic reactions. The modeling results further confirmed that SMZ was more mobile than CIP in the saturated porous media under the two pH conditions tested.

Effects of Ionic Strength on SMZ and CIP Transport

Solution IS showed little effect on SMZ transport in the sand columns under two pH conditions (Figures 2- 4a and 2-4b). The breakthrough responses of SMZ for two ISs were similar for pH 5.6 and pH 9.5. This is probably due to the fact that, under the experimental conditions, electrostatic interactions (ionic exchange and/or columbic attraction) between the SMZ and the quartz sand were negligible, and thus there was no competition effect from the cations or anions in the electrolyte. In a laboratory batch study, Gao and Pederson (Gao and Pedersen 2005) also found that IS had no influence on SMZ sorption to clay particles under these pH conditions.

Similarly, solution pH showed almost no effect on CIP transport in the sand in the sand columns at pH 9.5 (Figure 2- 5a), suggesting that electrostatic interactions between the CIP (CIP^- , 83.4%) and the negatively charged quartz sand were negligible. Even when the electrolyte concentration increased, there was no competition effect

from the cations or anions. At pH 5.6, however, solution IS did show certain effect on CIP transport in the sand columns. Although no breakthrough was observed at the end of the experiment, layer extraction results showed about 5% of the retained CIP was mobilized from front 1cm layer to the next layer (Figure 2-5b) at high IS. This result confirmed that electrostatic interactions could play an important role in controlling CIP transport in saturated porous media under low pH conditions, where the CIP is cationic and/or zwitterionic.

Conclusions

Laboratory column experiments were conducted to examine the effects of solution chemistry (i.e., IS and pH) on the retention and transport of two antibiotics in saturated porous media. Our results indicated that: 1) SMZ was more mobile in saturated porous media than CIP; 2) Solution pH played an important role in controlling the transport of CIP, but showed little effect on the transport of SMZ under the experimental conditions tested; 3) Solution IS had little effects on SMZ transport under the two pH conditions (9.5 and 5.6) but slightly enhanced the mobility of the retained CIP in the sand column at pH 5.6; and 4) traditional solute transport model could be used to simulate the retention and transport of antibiotics in water-saturated porous media.

Table 2-1. Basic properties of sulfamethoxazole (SMZ) and ciprofloxacin (CIP), the pKa values are from Lucida et al. (2000) and Vazquez et al. (2001), respectively.

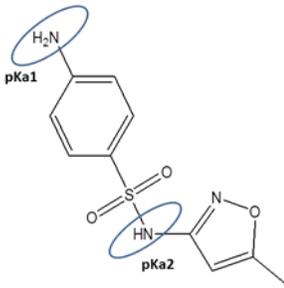
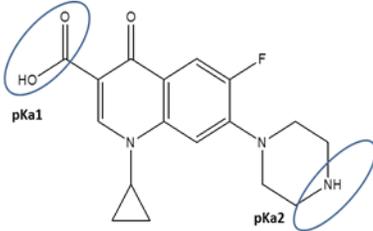
| <i>structure</i> | <i>MW</i> | <i>pKa</i> | <i>Speciation at pH 5.6</i> | <i>Speciation at pH 9.5</i> |
|--|-----------|------------------------|-----------------------------------|--------------------------------------|
| <p>SMZ</p>  | 253.3 | pKa1=1.7 pKa2=5.6 | Neutral (50%) and Anion (50%) | Anion (100%) |
| <p>CIP</p>  | 331.4 | pKa,1=6.2 pKa,2=8.8 | Cation (80%) and Zwitterion (20%) | Anion (83.4%) and Zwitterion (16.6%) |

Table 2-2. Summary of experimental conditions and model parameters (SMZ = sulfamethoxazole and CIP = ciprofloxacin)

| No. | Antibiotic | <i>IS</i> | <i>pH</i> | Antibiotic apply time | Flushing time | <i>R</i> | <i>k</i> |
|-----|------------|-----------|-----------|-----------------------|-----------------|-------------------------------------|----------|
| 1 | SMZ | 0 | 5.6 | 40 min (2 PV) | 80 min (4 PV) | 1.16 | 0 |
| 2 | SMZ | 0.1 | 5.6 | 40 min (2 PV) | 80min (4 PV) | 1.16 | 0 |
| 3 | SMZ | 0 | 9.5 | 40 min (2 PV) | 80 min (4 PV) | 1.16 | 0 |
| 4 | SMZ | 0.1 | 9.5 | 40 min (2 PV) | 80 min (4 PV) | 1.16 | 0 |
| 5 | CIP | 0 | 5.6 | 40 min (2 PV) | 80 min (4 PV) | Can not be fitted by proposed model | |
| 6 | CIP | 0 | 5.6 | 40 min (2 PV) | 240 min (12 PV) | Can not be fitted by proposed model | |
| 7 | CIP | 0 | 9.5 | 40 min (2 PV) | 80 min (4 PV) | 1.37 | 0.007 |
| 8 | CIP | 0.1 | 9.5 | 40 min (2 PV) | 80 min (4 PV) | 1.37 | 0.007 |

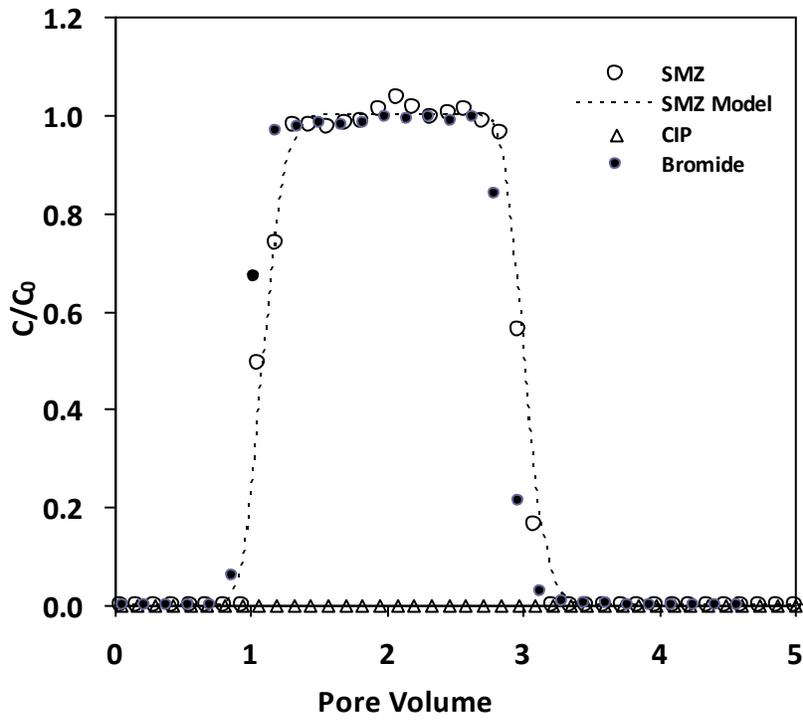


Figure 2-1. Transport of SMZ, CIP, and bromide in saturated sand columns under DI water conditions.

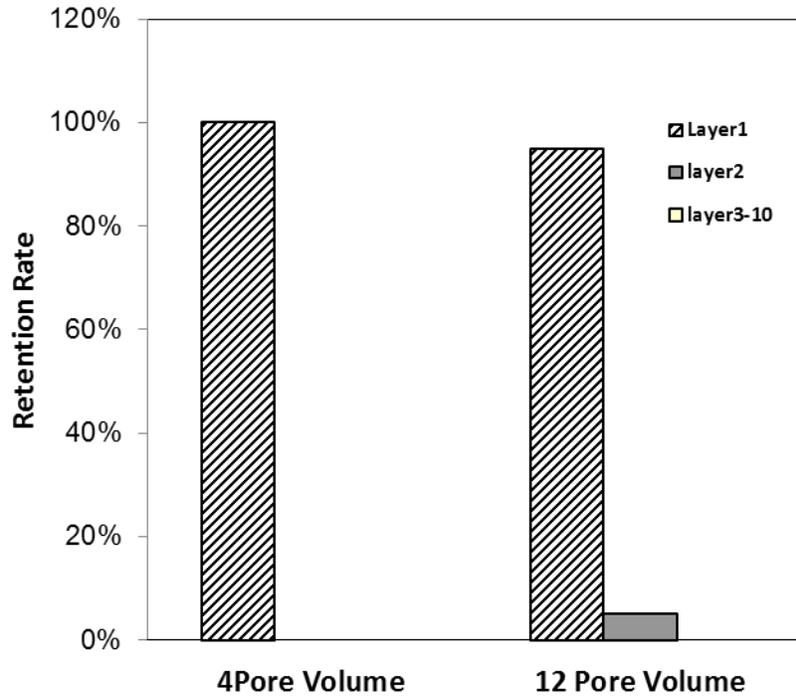


Figure 2-2. Distribution of retained CIP in the sand columns after extended DI water flushing. The column was disassembled into 10 pieces of 1-cm sand layer.

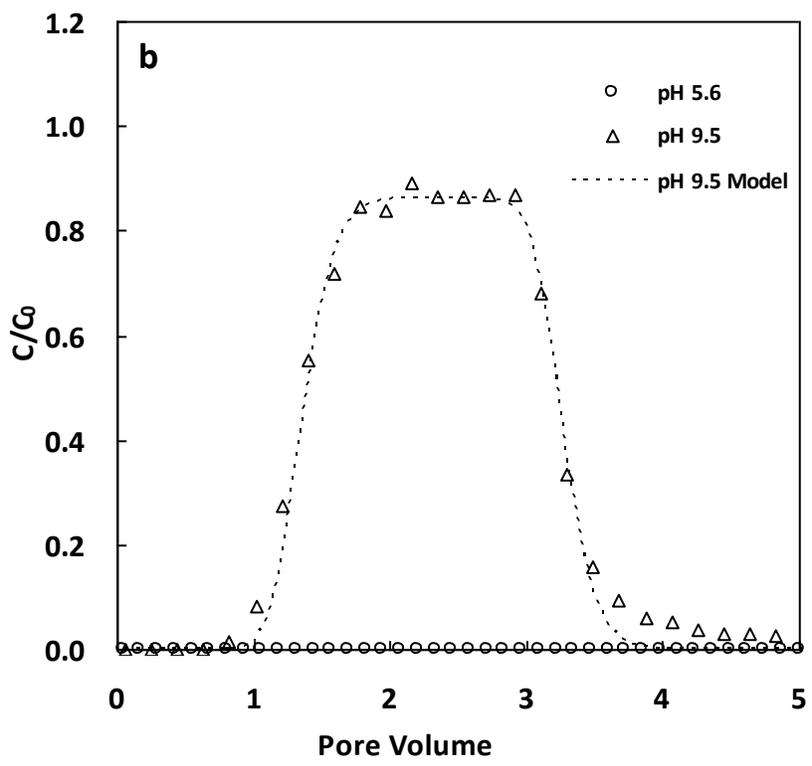
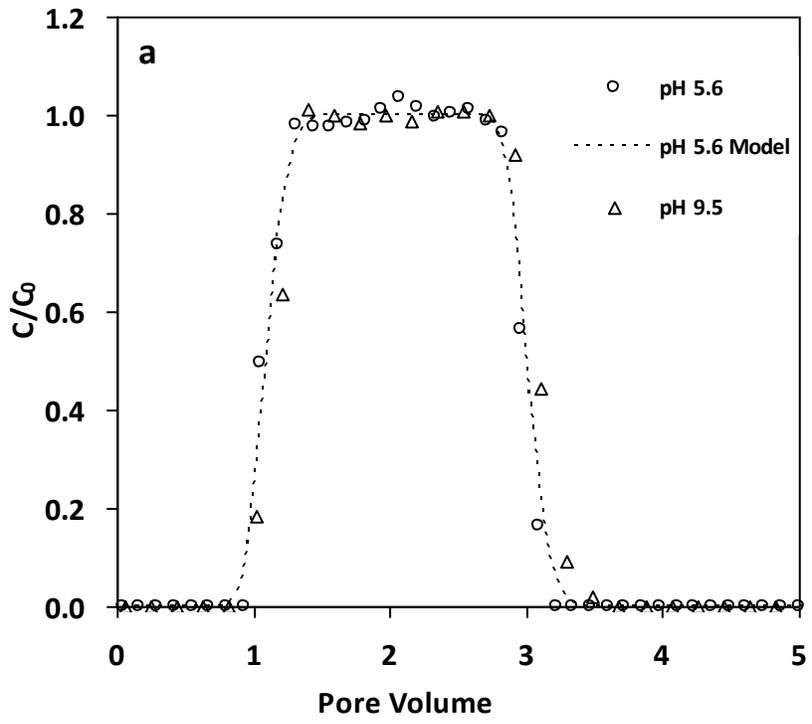


Figure 2-3. Effect of pH on the transport of SMZ and CIP in saturated sand columns

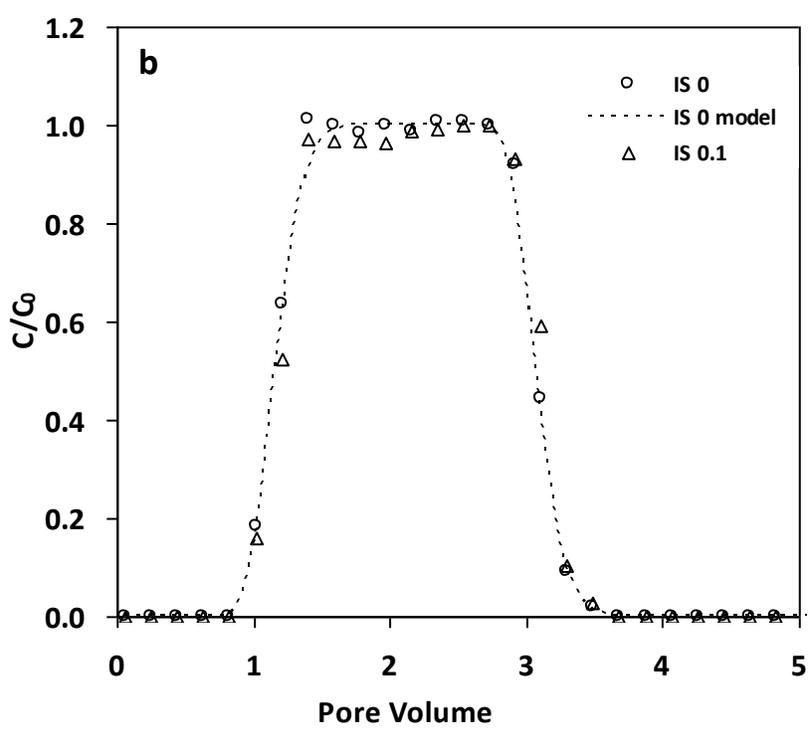
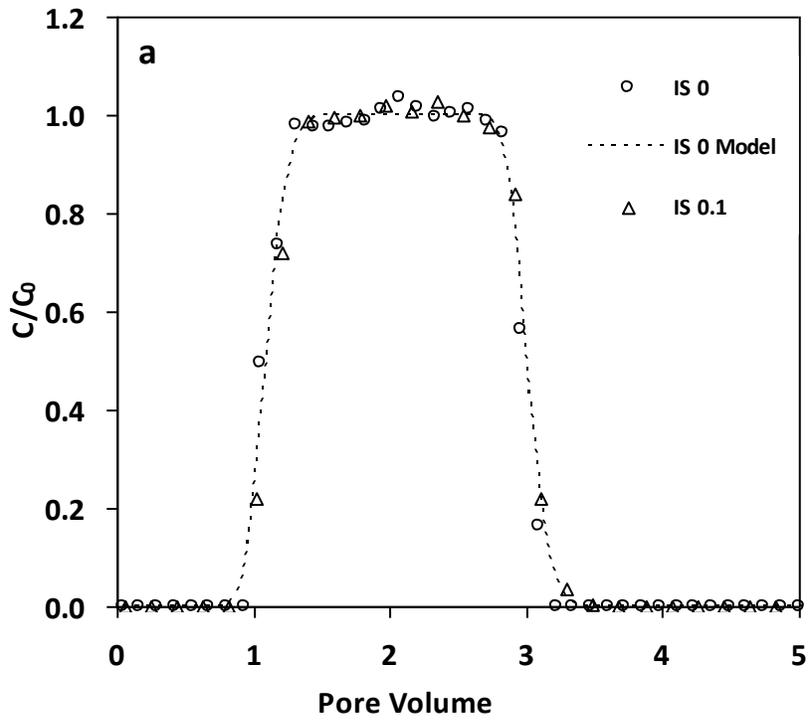


Figure 2-4. Effect of IS on the transport of SMZ in saturated sand columns at pH 5.6 and pH 9.5

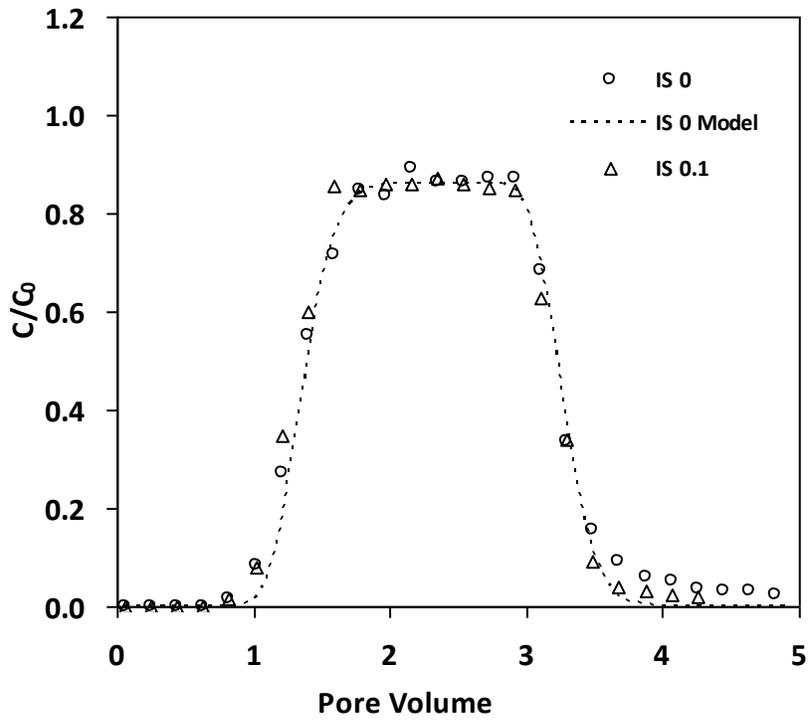


Figure 2-5. Effect of IS on the transport of CIP in saturated sand columns: (a) breakthrough curves at pH 9.5 and (b) distribution of retained CIP in the columns at pH 5.6

CHAPTER 3 INTERACTIONS OF CU AND CA WITH CIPROFLOXACIN SORPTION AND DESORPTION ONTO SATURATED POROUS MEDIA

Introduction

Antibiotics are widely used in health care and agricultural industries for the treatment and prevention of human and animal diseases. During the past decade, there has been growing concern about the release of antibiotics in the environment as these antibiotics are designed to be refractory to biodegradation and to act effectively even at low doses (Jones et al. 2001).

Ciprofloxacin (CIP) is one of the most widely prescribed fluoroquinolone antibiotic, also the main metabolite of enrofloxacin. The sources of CIP in the environment include land application of sewage sludge, wastewater irrigation, and disposing of expired pharmaceutical prescriptions (Golet et al. 2002), making CIP of increasingly environmental concern. It has been detected frequently in streams and groundwater (Kolpin et al. 2002; Barnes et al. 2004), with the concentrations ranging from ng L^{-1} to mg L^{-1} . Larsson et al (2007) reported that, in the wastewater treatment plant effluents from a pharmaceutical industry, CIP concentrations are 28 to 31 mg L^{-1} . CIP is known to be not readily biodegradable (Girardi et al. 2011) and has high sorption affinity onto soils (Nowara et al. 1997; Thiele-Bruhn et al. 2004; Vasudevan et al. 2009), so up to ppm levels of CIP can be accumulated in soils. Therefore, the soil can act as a reservoir of CIP and other antibiotics (Zorita et al. 2009). Hence it is important to examine the sorption and transport behaviors of CIP in soils.

Several mechanisms have been proposed for sorption of fluoroquinolone antibiotics onto soils. These include coulombic attraction (cation exchange and cation bridging) and surface complexation (Gu and Karthikeyan 2005; Otker and Akmehmet-Balcloglu 2005;

Trivedi and Vasudevan 2007). CIP sorption onto soils occurs via columbic attraction of its cationic amine moiety ($-\text{NH}_2^+$) to negatively-charged clay surface (Hari et al. 2005). Cation exchange occurs when cations in the solution replaces positively-charged CIP on solid surface. Cation bridging occurs when CIP is sorbed onto solid surface via columbic attraction of its carboxyl group ($-\text{COO}^-$) to sorbed cations (Nowara et al. 1997; Pei et al. 2009). In addition, surface complexation occurs between $-\text{COOH}$ group and surficial Fe/Al oxides in soils (Gu and Karthikeyan 2005; Hyun and Lee 2005). Hence, CIP can be sorbed onto soils via cation exchange, cation bridging and surface complexation (Vasudevan et al. 2009).

One approach to determine the sorption mechanisms for complex organic sorbate molecules with multiple functional groups such as CIP is through sorption of simple probe compounds with only one functional group (MacKay and Seremet 2008). In the case of CIP, structurally similar probe compounds of similar size are readily available. Sorption of the flumequine (FQ) with carboxylic group can help discern CIP complexation interactions with solid-phase or sorbed cations.

Most studies on CIP sorption didn't consider the influence of coexisting cations. Since CIP can exist as a cation, anion as well as zwitterion, it is important to consider the impact of cations. Metal cations in the environmental with relatively high concentration can impact CIP sorption by acting as competitor for its cationic moiety ($-\text{NH}_2^+$), acting as a bridge connecting its carboxyl group ($-\text{COO}^-$) to the negatively-charged site, or occupying the Fe/Al oxides sorption site to reduce its surface complexation with CIP (Wallis et al. 1996; Park et al. 2002). Few studies focused on the environmental fate of CIP with coexisting cations. Pei et al (2009) reported that Cu

increased CIP sorption onto both kaolinite and montmorillonite under certain pH range, but Ross and Riley (1992) demonstrated that Cu substantially increased the solubility and mobility of fluoroquinolone antibiotic. Although it has been demonstrated many cations have complexation ability to CIP (Turel et al. 1996), past research tested only copper. Therefore, it is necessary to investigate the impacts of other metal cations on CIP sorption and transport in the environment.

Fe/Al hydroxides are important mineral components in soils, especially in highly weathered soils (Essington 2003). Even present in limited quantities in soils, they are the major sorption sites for both organic and inorganic contaminants as they contain highly reactive surfaces (Huang et al. 1977; Violante et al. 2003). Fe/Al hydroxides can develop both positive and negative surface charge depending on solution pH. Hence, these minerals can exert a profound influence on the fate and behavior of contaminants in the soil. Sorption studies with pure minerals based on Fourier transform infrared (FTIR) spectroscopy showed that the carboxylic acid group participates CIP sorption onto Fe/Al oxides (Gu and Karthikeyan 2005). To our knowledge, few investigations looked at the sorption of fluoroquinolone antimicrobials on Fe/Al oxides under the influence of coexisting cations (Pei et al. 2009; Guaita et al. 2011).

In present study I hypothesized that both sand surface characteristics and the co-existence of divalent metal cations were the key factors controlling CIP sorption onto quartz sand. To test the hypothesis, batch experiments were conducted to compare the sorption behavior of CIP with two sands and two divalent metals. The goal of this study was to determine the mechanisms governing the sorption mechanism of CIP when co-existing with divalent metals in solution. Our specific objectives were to: 1) evaluate the

effects of sand surface metal oxides on CIP sorption; 2) evaluate the effects of divalent metals Cu and Ca on CIP sorption; and 3) evaluate the contribution of carboxylic and amine functional groups on CIP sorption onto sand.

Materials and Methods

Materials

CIP (ACS 85721-33-1) was purchased from Applichem (Darmstadt, Germany). Its chemical structure and basic information are in Table 3-1. All other chemicals were of analytical grades from Fisher Scientific (Pittsburgh, PA). CIP stock solution was prepared in DI water at a concentration of 40 mg L^{-1} . The stock solutions were stored at 4°C in darkness. The solutions were prepared in deionized (DI) water and glassware was acid-washed before use.

Quartz sand (45/30) from Standard Sand & Silica Co. (Davenport, FL) was sieved to a size range of 0.5-0.6 mm. To remove metal oxides on the sand surface, the native sand was first washed with tap and DI water, and then heated in 70% nitric acid at 90°C for 5 h. The sand was then washed with DI water to remove the acid and was referred to as clean sand. The sand has been used for column experiment, to better understand its interactions with CIP during transport; its sorption behaviors were investigated.

CIP Sorption onto Sand

CIP sorption isotherms onto native and clean sand were conducted using 50 mL polytetrafluoroethylene centrifuge tubes. Each vessel was filled with 3.00 g of sand and 30 mL of CIP at 9 different concentrations: 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.5 and 5.0 mg L^{-1} . The vessels were shaken for 24 h. Preliminary experiment showed that it reached equilibrium within 24 h (data not shown). The suspensions were centrifuged at

5,000 g for 10 min to separate the solids from the liquid phase. Aliquots of supernatant were withdrawn to determine CIP concentrations in solution using HPLC (Waters 2695, Milford, MA). Solid phase concentrations were obtained through mass balance calculations.

The impact of preloading Cu and Ca onto sand on CIP sorption by sand was investigated by shaking 3.00 g of sand with 30 mL of 1 mM CuCl_2 or CaCl_2 in a 50 mL centrifuge tube on a shaker for 24 h at room temperature. The Cu- or Ca-loaded sand was washed with DI water 5 times to remove soluble Ca and Cu. The sorbed Ca or Cu on the sand was analyzed by ICP-AES (Plasma 3200, Perkin-Elmer Crop, MA) after digesting with $\text{HNO}_3/\text{H}_2\text{O}_2$ hot-block digestion procedure (EPA). The Cu/Ca pre-loaded sand was then shaken with 30 mL of 1.0 mg L^{-1} of CIP for 24 h. The suspensions were then centrifuged and the supernatants were collected for CIP analysis using HPLC.

The impact of coexisting Cu and Ca on CIP sorption by sand was investigated by shaking vessels filled with 3.00 g of sand and 30 mL of 1.0 mg L^{-1} CIP at 6 different Cu:CIP or Ca:CIP molar ratios: 1, 10, 100, 500, 1,000, and 1,500. Different cations exist in soil solution so the complexation effect of these cations may add up. Some cations like Fe with extremely high complexation ability can be equivalent to much higher concentration of low complexation ability cations like Ca (Turel et al. 1996). To better understand the total effect of all the cations high concentration cations were also used. The vessels were shaken for 24 h and centrifuged at 5,000 g for 10 min to separate the solids from the liquid phase. Aliquots of supernatant were withdrawn for CIP analysis using HPLC. Solid phase concentrations were obtained through mass balance calculations.

Flumequine Sorption onto Sand

Since flumequine (FQ) contains a carboxyl group ($-\text{COOH}$) similar to CIP, it was used to investigate if the carboxyl group was involved in CIP sorption onto sand. It was done by shaking vessels filled with 3.00 g of sand and 30 mL of 3.3 mg L^{-1} CIP or 2.6 mg L^{-1} FQ (equal molar concentration at 0.01 mM) in DI water, and 1 mM CaCl_2 or CuCl_2 solution. The vessels were shaken for 24 h and centrifuged at 5,000 g for 10 min to separate the solids from the liquid phase. Aliquots of supernatant were withdrawn for CIP and FQ analysis using HPLC. Solid phase concentrations were obtained through mass balance calculations.

Analysis of Antibiotics and Cu and Ca

The analysis of CIP and FQ was carried out using a HPLC (Waters 2695, Milford, MA) equipped with a Nova-Pak C18 column (150 mm \times 3.9 mm, Waters Millipore). The mobile phase consisted of acetonitrile and 0.5% phosphoric acid at a ratio of 15:85. A fluorescence detector (Waters 2475) was used to detect CIP and FQ with the excitation and emission wavelengths at 278 and 445 nm, respectively. The CIP detection limit was $5 \text{ } \mu\text{g L}^{-1}$ and the linear range was 50–25,000 $\text{ } \mu\text{g L}^{-1}$ with correlation coefficients (R^2) > 0.99. The FQ detection limit was $20 \text{ } \mu\text{g L}^{-1}$ and the linear range was 100–10,000 $\text{ } \mu\text{g L}^{-1}$ with correlation coefficients (R^2) > 0.99. Concentrations of Ca or Cu were analyzed by ICP-AES (Plasma 3200, Perkin-Elmer Crop, MA). The detection limit of this method for Cu and Ca was 0.02 mg L^{-1} and 0.1 mg L^{-1} respectively.

Results and Discussion

CIP Species in Solution

Since CIP species impact its interactions with Cu, Ca and sand, it is important to know its speciation in solution. CIP can exist in three forms (cationic, zwitterionic, and

anionic forms) in DI water with two proton-binding sites (carboxyl and piperazinyl groups; Table 3-1). Under experiment condition of this study at pH 5.6, CIP existed as cationic form (80%) and zwitterionic form (20%).

In comparison, >99% Cu and Ca were present as free cations (Visual-MINTEQ). When CIP coexists with metal cations in solution, it can complex with Cu or Ca via its carboxyl and keto groups to form metal-CIP or metal-CIP₂ complexes. In this study, since ratios of Cu or Ca to CIP were much greater than 1, so only 1:1 metal-CIP was considered (Li et al. 1994; Turel et al. 1996).



Based on the complexation stability constants of Cu and Ca with CIP (log k = 14.7 and 11.3; Table 3-1b), CIP interacted with Cu more strongly than Ca. Since fluorescence quenching ability can be used to indicate metal's complexation ability (Park et al. 2007), the complexation ability of Cu or Ca was further tested by fluorescence quenching experiment. Assuming the fluorescence intensity of 200 µg L⁻¹ CIP in DI water was 100, the fluorescence intensity in 0.1 mM Ca and 0.1 mM Cu were 67 and 42 (data not shown), indicating both Cu and Ca were effective in fluorescence quenching, with Cu being 60% stronger than Ca. Compared to Cu/Ca, the complexation ability of CIP to Fe/Al is much stronger, forming Fe/Al-CIP₃ (log k = 46.9 and 43.6; Table 3-1b).

Characteristics of Sand

Compared to soils, sand is more homogenous and much simpler with limited functional groups. So sand is a good material to investigate part of the sorption mechanisms of organic contaminants. However, even with simple materials like sand,

the sorption mechanisms of CIP onto sand are complicated. Clean sand is composed of mostly SiO_2 , with its sorption capacity coming from negatively-charged broken edges ($\equiv\text{SiO}^-$) (Essington 2003). In addition to $\equiv\text{SiO}^-$, native sand was coated with Fe/Al oxides (Table 3-3).

At pH 5.6, both native and clean sand carried negative charges with PZC (point of zero charge) being 5.0-5.1 (Table 3-3). The PZC for Fe/Al oxides is 10 (Essington 2003) so locally the broken edges of Fe/Al oxides carried positive charges ($\equiv\text{Fe/AlOH}^+$). In addition, the Fe/Al oxides on the broken edges with high Gibbs free energy are more reactive in surface complexation with CIP (Yost et al. 1990; Molis et al. 2000; Duckworth and Martin 2001). Trivedi et al.(2001) demonstrated that sorption of Cu as a transition metal is more likely to bind to high affinity site on goethite, while Ca as an alkaline earth metal bind to low affinity site (Trivedi et al. 2001; Violante et al. 2003).

CIP Sorption Isotherm onto Clean and Native Sand

Both clean sand and native sand were able to sorb CIP, but their ability differed substantially. The sorption of CIP onto native sand was significantly higher than clean sand. CIP sorption data fit well with Langmuir equation ($R^2 = 0.9986-0.9998$; data Show as Appendix), suggestive of monolayer sorption. The best-fit value of maximum sorption capacity from Langmuir model for native sand was 50 mg kg^{-1} , which was 10 times greater than that of clean sand 5 mg kg^{-1} . Moreover, the affinity of binding sites calculated from Langmuir model on the native sand ($1.95 \text{ L } \mu\text{g}^{-1}$) was 6 times greater than that on the clean sand ($0.33 \text{ L } \mu\text{g}^{-1}$).

Though clean sand mainly consists of pure silica oxides, it still sorbed some CIP, which was probably attributed to columbic interaction. At pH of 5.6, CIP was ~80%

positively charged and 20% zwitterionic (pka1=6.2 and pka2 = 8.8; Table 3-1). Hence, CIP sorption onto clean sand could be attributed to columbic attraction between positively-charged CIP amine groups ($-\text{NH}_2^+$) and negatively-charged sand surfaces ($\equiv\text{SiO}^-$, Table 3-3).

Compared to clean sand, native sand not only provided more sorption sites but also had higher sorption affinity. This was mainly attributed to Fe/Al oxides on the surface ($\equiv\text{Fe/AlO}^+$), which amounted to 167 and 1,087 mg kg^{-1} (Table 3-4). Its surface characteristics based on SEM was consistent with the chemical analysis, EDS spectra also indicated that the native sand contained substantial amounts of Fe/Al (data Show as Appendix). Previous study indicate surface complexation of Fe/Al oxides with CIP was with its carboxylic acid group (Gu and Karthikeyan 2005). Assuming all the extra sorption capacity of native sand was contributed to Fe/Al oxides with equal sorption ability, their sorption capacity was calculated at 33g kg^{-1} . This suggests the sorption capacity of Al/Fe oxides on sand surface is comparable to that of the pure phase (Gu and Karthikeyan 2005), with sorption capacity of Al oxides at 21.8 g kg^{-1} and Fe oxides 13.5 g kg^{-1} .

Based on the above discussion, CIP sorption onto sand in DI water was mainly controlled by two mechanisms (Table 3-2): 1) columbic attraction between cationic amine groups of CIP and negatively-charged sand surface (Hari et al. 2005), and 2) surface complexation between anionic carboxyl groups of CIP and Fe/Al oxides on native sand (Gu and Karthikeyan 2005).



CIP Sorption onto Sand Preloaded with Cu and Ca

To better understand the sorption mechanisms of CIP onto sand, the sand was preloaded with Cu or Ca. The amounts of Cu sorbed onto native and clean sand were 8 and 1 mg kg⁻¹ whereas those of Ca were below the detection limit of ICP (data not shown). The higher sorption ability of Cu than Ca for both sands could be explained by Cu's higher ionic potential (Z/r , 0.022 vs. 0.009; Essington, 2003) than Ca in aqueous solutions. The higher sorption of Cu onto native sand than clean sand was mostly attributed to its higher cation exchange capacity (CEC = 0.28 vs. 0.05 cmol kg⁻¹; Table 3-1c). However, the amount of Cu sorbed by the sand was much lower than that predicted based on CEC. Also although the net Fe/Al oxides minerals are positively charged, they still could adsorb cations to form inner sphere complexes at low pH values (Violante et al. 2003).

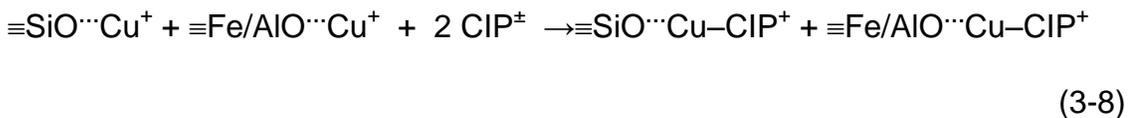
As expected, preloading Cu and Ca onto sand surface increased its CIP sorption (Table 3-4). It is known that CIP forms Cu-CIP and Ca-CIP complex in solution (Turel et al. 1996). The increased CIP sorption onto Cu-loaded sand was through cation bridging effect either forming metal-complex at strong affinity sites or via Columbia interactions at weak affinity sites. This is supported by Guaita et al (2011) who examined the impact of Cu on flumequine sorption (a fluoroquinolone antibiotic). They found Cu increases flumequine accumulation onto soil via formation of Cu-flumequine ternary surface complex. A separate study by Pei et al. (2009) on CIP and Cu cosorption onto clays has similar result. They attributed the stronger affinity of CIP-Cu complex onto sand than CIP species to Cu bridging effect between the clay surface and CIP (Eqs.3- 1 and 3-2).

After being saturated with Cu^{2+} , $\equiv\text{SiO}^-$ or $\equiv\text{Fe}/\text{AlO}^-$ was most likely present as $\equiv\text{SiO}\cdots\text{Cu}^+$ or $\equiv\text{Fe}/\text{AlO}\cdots\text{Cu}^+$ (Eqs. 3-5 & 3-7). CIP was then sorbed onto Cu-preloaded sand (Table 3-2) via Cu^{2+} bridging (Eqs. 3-6&3-8).

Clean sand



Native sand



Since both clean and native sand were able to sorb Cu (1 and 8 ppm), preloading Cu increased CIP sorption by 0.58 and 2.11 mg kg^{-1} for clean and native sand respectively (Table 3-2). Assuming Cu:CIP ratio at 1:1, then based on the Cu concentrations preloaded on sand, the projected CIP concentrations were 5.2 and 41.8 mg kg^{-1} . However, the actual sorbed CIP was much lower. It was possible only part of the Cu sorbed was suitable for CIP sorption.

Based on the Cu concentrations in the solution after CIP sorption onto Cu-preloaded sand, 0.15 and 0.8 mg kg^{-1} Cu was stripped off the clean and native sand surface (data not shown). It was possible that some Cu, which was sorbed onto sites with low affinity, was stripped off the sand surface after it complexed with CIP and came into solution. Only those Cu sorbed onto sites with high affinity stayed on sand surface. This results were in agreement of Pei et al (2009) who observed ligand-promoted dissolution of soil surface cations in the presence of CIP.

Compared to Cu, preloaded Ca had different impact on CIP sorption. No impact was observed on clean sand as little Ca was sorbed onto clean sand; however preloading Ca onto native sand increased its CIP sorption by 2.6 mg kg^{-1} , which was higher than that of Cu (2.11 mg kg^{-1}). Though under detection, some Ca was sorbed onto native sand. The additional CIP was sorbed onto sand via Ca bridging similar to Cu. However, since CIP has weaker complexation with Ca than Cu, less CIP-Ca was stripped off sand surface than CIP-Cu ($\log k = 14.7$ and 11.3 ; Table 3-1b).

Premixing Cu and Ca with CIP Reduced CIP Sorption onto Clean Sand

To further test the above hypothesis, excess amounts of Ca/Cu was mixed with CIP and then mixed with sand. With increasing cation concentrations, both Cu and Ca decreased CIP sorption onto clean sand (Figure 3-1 A), with Cu being more effective than Ca.

Since excess amounts of Ca^{2+} and Cu^{2+} were mixed with CIP, CIP was mostly present as Ca-CIP^+ and Cu-CIP^+ in solution (Table 3-2; Eqs. 3-1 & 2). Since the major reactive sites on sand were $\equiv\text{SiO}^-$, the increasing presence of Ca/Cu in solution would mainly compete with Ca-CIP^+ and Cu-CIP^+ for the negatively charged $\equiv\text{SiO}^-$ sites. With increasing Ca/Cu concentrations (up to 30 mg L^{-1}), Ca/Cu out competed Ca-CIP^+ and Cu-CIP^+ for the sorption onto $\equiv\text{SiO}^-$, thereby decreasing CIP sorption onto sand (Table 3-2). However, even at 30 mg L^{-1} Ca, still some CIP remained on the sand. In comparison, at that ratio, Cu was able to strip almost all CIP off the sand (Figure 3-1A), reflecting Cu as a transition metal of higher charge density was more effective in CIP desorption via complexation.

Premixing CIP with Cu and Ca changed CIP sorption onto native sand

Similar to clean sand, with increasing Cu/Ca concentrations in solution, they outcompeted Ca–CIP⁺ and Cu–CIP⁺ for negative sorption sites on sand surface, reducing CIP sorption onto sand. However, in native sand, besides ≡SiO⁻ sites, ≡Fe/AlO⁻ sites were also present. Though in limited quantity, they were more effective in CIP sorption than ≡SiO⁻ sites (7.00 vs 3.05 mg kg⁻¹; Table 3-2). So, with increasing Cu/Ca concentrations in solution, initially they increased CIP sorption, which may result from cation bridging effect of ≡Fe/AlO...Cu–CIP and ≡Fe/AlO...Ca–CIP (Eq. 3-5 to 3-8) (Table 3-2). However, when cation concentration continued to increase, CIP sorption onto sand significantly decreased (Figure 3-1B). Similar to the effect of Ca and Cu on CIP sorption onto clean sand, the increasing cations in the solution probably stripped CIP complexes (Cu–CIP⁺ or Ca–CIP⁺) off the native sand surface as they both carried positive charge, leaving Cu or Ca on sand surface. Under the experiment condition, the greater efficiency of Cu was due to its higher affinity to CIP and to sand surface than Ca (Table 3-1b) (Wallis et al. 1996; Upadhyay et al. 2006).

Since cations sorbed on native sand surface acted as a bridge and facilitated CIP sorption while cations in the solution phase competed with CIP for sorption onto sand. The ratio of cation concentrations on the sand surface and solution should be directly related to CIP sorption. The K_d of CIP had linear correlation with the ratio of cation in the sand to that in solution (Cu at R²=0.9566, and Ca at R²=0.9055). This confirmed that cations in the environment could either facilitate or impede CIP sorption depending on whether they are on solid or solution phase.

Comparison of CIP Sorption with Probe Compound Flumequine

In our experiment, the carboxylic group of CIP interacted with Cu/Ca and Fe/Al oxides on sand surface. It is possible that other fluoroquinolone antibiotics with similar functional group have similar behavior. I used FQ with fundamental fluoroquinolone antibiotics structure to further test this hypothesis.

The molar ratio of Cu or Ca to CIP or FQ in the solution was 100:1, so most of the CIP or FQ should be complexed with Cu or Ca and excess amount of Cu or Ca was present in the solution.

In the absence of Cu or Ca, 1.58 mg kg⁻¹ of CIP was sorbed onto clean sand. The presence of Ca reduced CIP sorption by ~50% to 0.83 mg kg⁻¹ whereas Cu completely inhibited CIP sorption onto clean sand. Both Ca and Cu competed with CIP for sorption onto ≡SiO⁻ sites as electrostatic interaction was the main driven force for CIP sorption (Figure 3- 2). Compared to CIP, FQ structure lacks positive charge so reduced amount of CIP was sorbed onto clean sand at ~11% or 0.20 mg kg⁻¹. Compared to CIP, the reduced sorption of FQ onto sand was probably due to its lack of positive charge as clean sand surface was negatively charged. Similar to CIP sorption, both Ca and Cu reduced FQ sorption with Cu completely inhibiting FQ sorption.

In the absence of Cu or Ca, native sand was more effective than clean sand, sorbing 11.7 mg kg⁻¹ CIP. However, the presence of Ca increased its sorption to 16.7 mg kg⁻¹ whereas Cu reduced its sorption to 1.80 mg kg⁻¹. Similar data were obtained for FQ, which indicate that its carboxyl group was mostly responsible for their sorption of CIP or FQ. The data also imply that native sand was more effective in sorbing CIP or FQ than CIP–Cu or FQ–Cu complexes. Since Ca has weak complexation ability with

CIP or FQ, it helped their sorption via cation bridging (Park et al. 2000). This is consistent with the study of enrofloxacin and other important quinolone antibiotic containing carboxylic groups (Nowara et al. 1997). They also observed the high affinity between quinolone antibiotics and metal oxides, further demonstrating the importance of the carboxyl group in quinolone antibiotic in controlling its behaviors in the environment.

Environmental Implication

Although CIP has been demonstrated to have complexation ability with many cations, only the environmental influence of Cu was investigated in previous study (Pei et al. 2009; Guaita et al. 2011). Also cation concentration as high as several hundred mg kg⁻¹ has been frequently reported in surface soil (Muchuweti et al. 2006), hence, the impact of cations at high concentrations should also be included to better understand the environmental behavior of CIP. In this study I demonstrated both Ca and Cu impacted CIP sorption onto sand. This indicated other metals with similar complexation ability with CIP should not be overlooked when present in the system. These cations sorbed on sand surface can decrease CIP mobility by increase CIP sorption while cations in solution can promote CIP mobility by reducing CIP sorption. In addition, due to Cu's strong complexation ability with CIP, it was more effective in stripping CIP off sand surface.

Recent study indicated after complexation with cations, CIP can be active over time and inhibit microbial activities in both solution and solid phase (Girardi et al. 2011). For CIP sorption experiment using Ca to adjust ionic strength (Zhang and Dong 2008; Wang et al. 2009), it may not reflect the CIP sorption behavior at equilibrium as Ca can not only complex with CIP but also compete with CIP on sorption sites in soils.

Conclusions

I examined the effects of divalent metals Ca and Cu on CIP sorption on clean sand native sand. Our results indicated that: 1) Fe/Al oxides on native sand surface was responsible for CIP sorption; 2) Fe/Al oxides played an important role in cation bridging effect of Cu and Ca; 3) Both Cu and Ca decreased CIP sorption onto clean sand; and 4) Both Cu and Ca promoted CIP sorption onto native sand, but at higher concentrations, they decreased CIP sorption. Our research demonstrated the importance of cations such as Cu and Ca in controlling the fate and transport of CIP in the environment.

Table 3-1. Basic properties of ciprofloxacin (CIP)

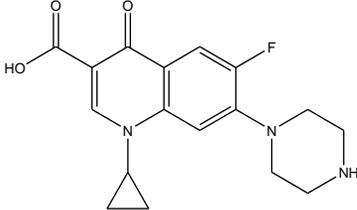
| Structure | MW | pKa | Species at pH 5.6 |
|--|-------|----------------------|----------------------------------|
|  <p>The chemical structure of ciprofloxacin consists of a central quinolone ring system. At position 2, there is a cyclopropyl group. At position 3, there is a carboxylic acid group (-COOH). At position 4, there is a piperazine ring. At position 6, there is a fluorine atom. The quinolone ring has a carbonyl group at position 1 and a double bond between positions 2 and 3.</p> | 331.4 | pKa1=6.2 pKa2=8.8 | Cation (80%) Zwitterion (20%) |

Table 3-2. Stability constant (log k) of CIP with Cu, Ca, Fe and Al (1996)

| | Ca | Cu | Fe | Al |
|---------------------|------|------|------|------|
| M(CIP) | 11.3 | 14.7 | | |
| M(CIP) ₂ | | 28.5 | | 29.3 |
| M(CIP) ₃ | | | 46.9 | 43.3 |

Table 3-3. Basic property of sand

| | Particle size (µm) | CEC (cmol kg ⁻¹) | ZPC* | Total Fe (mg kg ⁻¹) | Total Al (mg kg ⁻¹) |
|-------------|--------------------|------------------------------|------|---------------------------------|---------------------------------|
| Clean Sand | 4.5-5.5 | 0.05 | 5.0 | | |
| Native Sand | 4.5-5.5 | 0.28 | 5.1 | 167 | 1,087 |

* ZPC = zero point charge

Table 3-4. The impact of Ca or Cu on CIP sorption onto clean and native sand (3 g sand with 1 mg L⁻¹ CIP for 24 h)

| Treatment | CIP sorbed (mg kg ⁻¹) | | CIP in solution (µg L ⁻¹) | | Potential sorption mechanisms | |
|--|-----------------------------------|-----------|---------------------------------------|--------|---|---|
| | Clean | Native | Clean | Native | Clean | Native |
| DI water | 3.05±0.10 | 7.00±0.17 | 695 | 300 | ≡SiO ^{•••} CIP | ≡Fe/AIO–CIP ≡SiO ^{•••} CIP |
| ≡Si/Fe/AIO ⁻ –Ca ²⁺ + CIP | 2.98±0.06 | 9.60±0.82 | 702 | 40 | ≡SiO ^{•••} CIP ≡SiO ^{•••} Ca-CIP | ≡SiO ^{•••} CIP ≡SiO ^{•••} Ca–CIP ≡Fe/AIO–CIP ≡Fe/AIO ^{•••} Ca/Cu–CIP |
| ≡Si/Fe/AIO ⁻ –Cu ²⁺ + CIP | 3.63±0.03 | 9.11±0.22 | 637 | 89 | ≡SiO ^{•••} CIP ≡SiO ^{•••} Cu-CIP | ≡SiO ^{•••} CIP ≡SiO ^{•••} Cu–CIP ≡Fe/AIO–CIP ≡Fe/AIO ^{•••} Cu–CIP |
| ≡Si/Fe/AIO ⁻ –Ca ²⁺ + Ca ²⁺ –CIP | 2.90±0.07 | 9.57±1.4 | 710 | 44 | ≡SiO ^{•••} CIP ≡SiO ^{•••} Ca-CIP | ≡SiO ^{•••} CIP ≡SiO ^{•••} Ca–CIP ≡Fe/AIO–CIP ≡Fe/AIO ^{•••} Ca-CIP |
| ≡Si/Fe/AIO ⁻ –Cu ²⁺ + Cu ²⁺ –CIP | 4.09±0.08 | 9.25±0.84 | 591 | 79 | ≡SiO ^{•••} CIP ≡SiO ^{•••} Cu-CIP | ≡SiO ^{•••} CIP ≡SiO ^{•••} Cu–CIP ≡Fe/AIO ^{•••} CIP ≡Fe/AIO ^{•••} Cu–CIP |

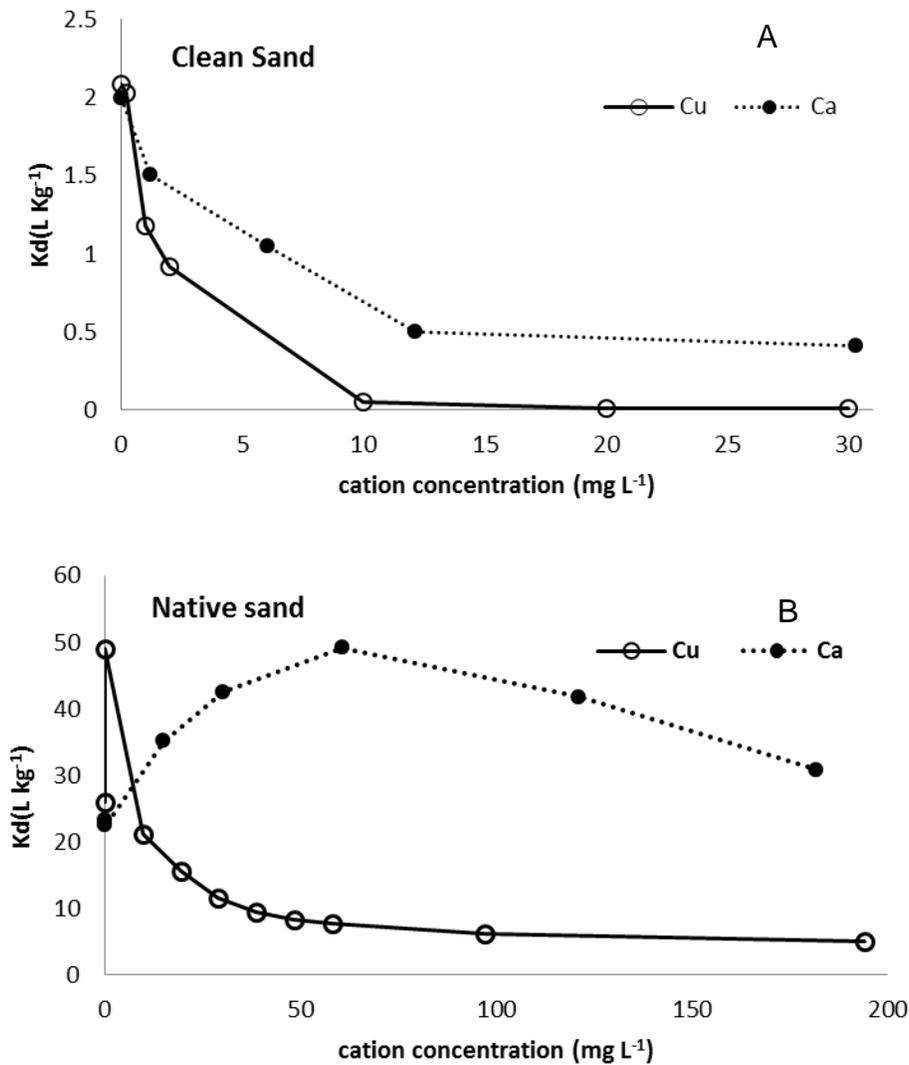


Figure 3-1. CIP sorption coefficient K_d^* by clean and native sand at 1 mg L^{-1} CIP different cation concentrations. A) clean sand, and B) native sand $*K_d = C_s/C_w$; where C_s is solid phase CIP concentration; C_w is the solution phase CIP concentration.

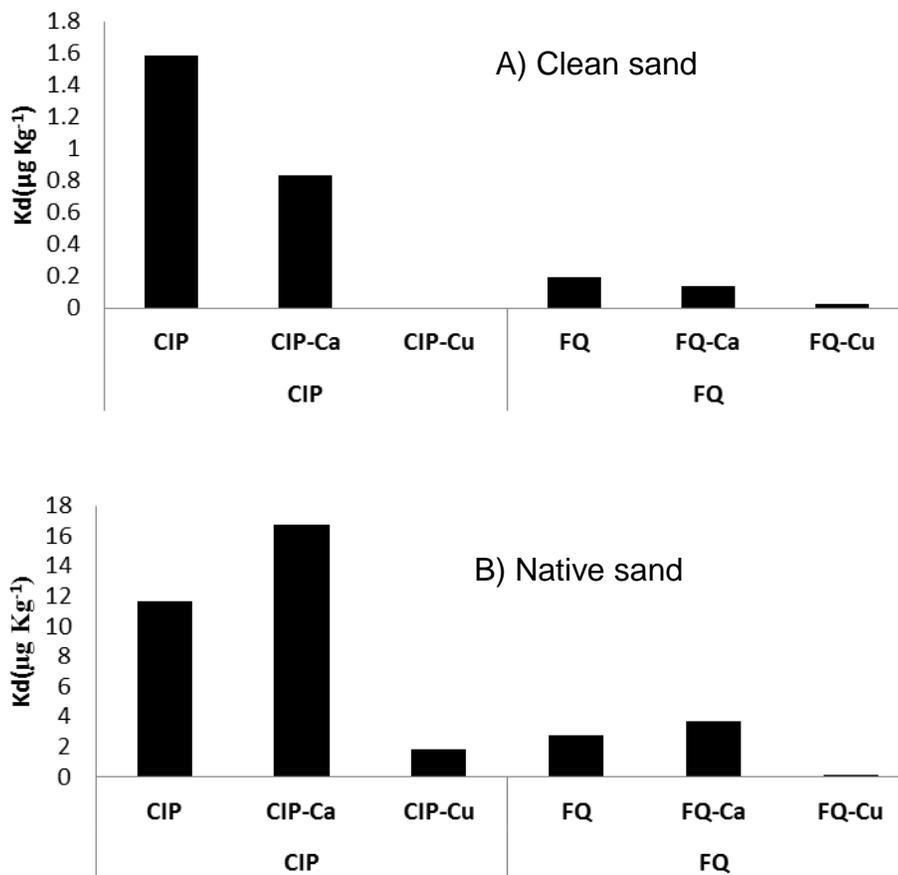


Figure 3-2. Sorption ability of sand of 0.01 mmol CIP or FQ in DI water, 1 mmol CaCl₂ or CuCl₂ solution. A) clean sand, and B) native sand

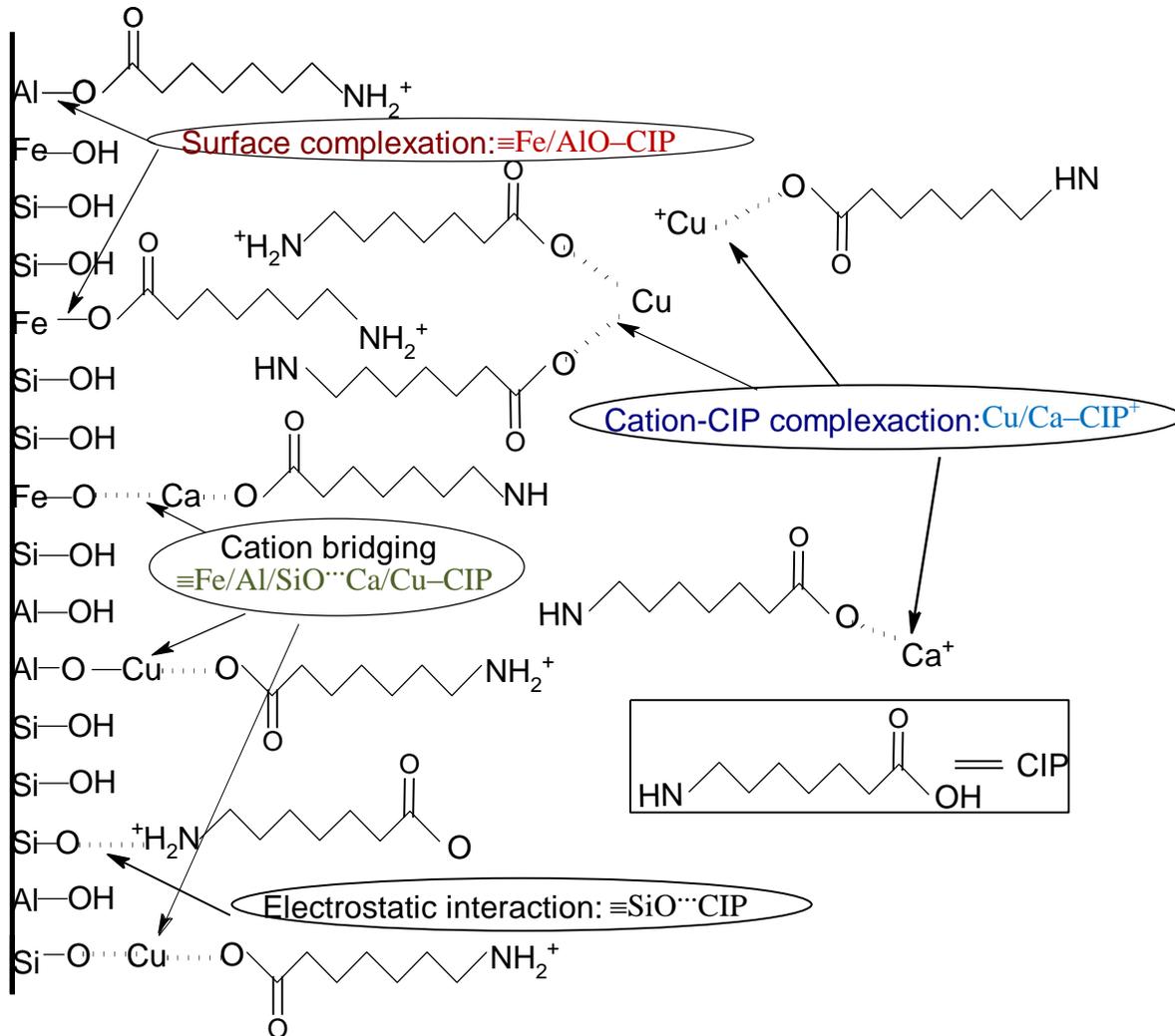


Figure 3-3. Possible interactions in the system

CHAPTER 4 INFLUENCE OF CU AND CA ON CIPROFLOXACIN TRANSPORT IN SATURATED POROUS MEDIA

Introduction

Ciprofloxacin (CIP), one of the most widely prescribed fluoroquinolones (FQs) antibiotics, is widely used in health care and agricultural industries to treat and prevent human and animal diseases. It is designed to be refractory to biodegradation and to act effectively even at low doses (Jones et al. 2001), so it is persistent in the environment and imposes a risk to the ecosystems. CIP has been frequently detected in streams and groundwater (Kolpin et al. 2002; Barnes et al. 2004), with the concentrations ranging from ng L^{-1} to mg L^{-1} (Larsson et al. 2007). CIP is known to be not readily biodegradable (Girardi et al. 2011) and has high sorption affinity onto soils (Nowara et al. 1997; Thiele-Bruhn et al. 2004; Vasudevan et al. 2009), so up to ppm levels of CIP can be accumulated in soils. Therefore, the soil can act as a reservoir of CIP and other antibiotics (Zorita et al. 2009). Hence, it is important to examine the fate and transport behaviors of CIP in soil and water system.

The ability of FQs to form complexes with various metal cations is important for their antibiotic activity (Turel et al. 1996), so metal cations are expected to influence their fate and transport in the environment. The high affinity of soil matrix for FQs has been attributed to their complexation with either exposed structural or exchangeable cations (Drakopoulos and Ioannou 1997; Nowara et al. 1997; Trivedi et al. 2001; Gu and Karthikeyan 2005; Zhang and Huang 2005), and the attraction of their protonized piperazine moiety (NH_2^+) to the negatively-charged mineral surface (Goyne et al. 2005). Interaction between FQs and soil minerals are universal even silica shows substantial sorption ability for FQs (Goyne et al. 2005). However, it should be noted that the

interaction between FQs and metal cations are reversible and controlled by the density of charge of metal cations (Turel et al. 1996; Aristilde and Sposito 2008). The impacts of metal cations on CIP sorption onto minerals have been studied in batch experiments (1992; Pei et al. 2009), however, little information is available on their impacts on CIP transport. Minerals like Fe/Al oxides can develop both positive and negative surface charge (Huang et al. 1977; Violante et al. 2003), even present in limited quantities in soils, they are the major sorption sites for CIP. Hence, metals in both aqueous and mineral phase greatly influence the fate and transport of CIP in porous media and soils.

This study was to provide insights into how metals influence CIP transport in porous media to better understand the chemodynamic behaviors of CIP in soils and receiving waters, which is essential for comprehensive assessment of their potential environmental risk. I hypothesized that both minerals on sand surface and the presence of metal cations were critical factors controlling CIP transport in saturated porous media. To test the hypothesis, column experiments using saturated quartz sand were conducted to compare the transport behavior of CIP in two sands and in presence of two metals. The specific objectives were to: 1) evaluate the effects of metal oxides on sand surface on CIP transport in porous media, 2) compare the effects of metals Cu and Ca on retention and transport of CIP in porous media; 3) compare the effects of metals Cu and Ca on mobilization of CIP presorbed on porous media; and 4) apply solute transport models to simulate the retention and transport of CIP in porous media.

Materials and Methods

Materials

Ciprofloxacin (ACS 85721-33-1) was purchased from Applichem (Darmstadt, Germany). Its chemical structure and basic information are presented in Chapter 3. Quartz sand treatment and basic information was same as chapter 3

Column Experiments

See Chapter 2 Modeling Antibiotic Transport in Saturated Porous Media section.

Impacts of Aqueous Ca and Cu on CIP Transport in Sand Media

To test the impacts of aqueous Ca and Cu on CIP transport in sand media, 0.2 mg L⁻¹ CIP (0.7 μ M) was mixed with DI water, 1 mM CaCl₂, or 1 mM CuCl₂. Once the outflow was stabilized, the experiment was then terminated. Effluent samples were collected from the top of the column with a fraction collector (IS-95 Interval Sampler, Spectrum Chromatography) during sample injection and column flushing to analyze CIP concentrations. All experiments were performed in duplicate.

To determine the impacts of Ca and Cu on CIP sorption onto sand, separate batch study with same CIP concentration and Ca or Cu solution was conducted using 50 mL polytetrafluoroethylene centrifuge tubes. Each vessel was filled with 3.00 g of sand and 30 mL of 0.7 μ M CIP with DI water, 1 mM CaCl₂ or 1mM CuCl₂. The vessels were shaken for 24 h. Preliminary experiment showed that it reached equilibrium within 24 h (data not shown). The suspensions were centrifuged at 5,000 g for 10 min to separate the solids from the liquid phase. Aliquots of supernatant were withdrawn to determine CIP concentrations, solid phase concentrations were obtained through mass balance calculations. The CIP concentration was determined by HPLC (Waters 2695, Milford, MA) equipped with a fluorescence detector (Waters 2475, Milford, MA). The

sorbed Ca or Cu on the sand was analyzed by ICP-MS (NexION 300, Perkin-Elmer Crop, MA) after digesting with HNO₃/H₂O₂ hot-block digestion procedure (EPA). The detection limits for Ca and Cu were 0.5 µg kg⁻¹ and 0.05 µg Kg⁻¹, respectively. Batch experiments were performed in triplicate.

Impacts of Aqueous Ca and Cu on Mobilization of CIP Presorbed onto Sand Media

In the previous experiment, CIP and Cu or Ca was transported simultaneously in the sand column. To determine the ability of aqueous Ca and Cu in mobilizing CIP in sand media, CIP was applied to the column at a 5 PV pulse, and then the column was flushed with DI water, 1 mM CaCl₂, or 1 mM CuCl₂. Experiment was terminated when out flow stabilized or after 100 PV. Effluent samples were collected from the top of the column with a fraction collector during flushing with Ca or Cu solution after CIP injection pulse to analyze CIP concentrations. All experiments were performed in duplicate.

Since Ca/Cu was ineffective in mobilizing CIP presorbed onto sand column, a separate column study was conducted. For each set of experiment, CIP was applied to the columns at a 5 PV pulse, and then the columns was flushed 5 PV of DI water, 1 mM CaCl₂, or 1 mM CuCl₂, respectively. At the end of flushing, the column was separated into 10 sections to determine the concentrations of retained CIP as a function of column depth. The sand was excavated under saturated conditions from top to bottom with a spatula in 1 cm increment and each increment was placed into a small vial with 4 mL of H₃PO₄-KH₂PO₄ buffer and acetonitrile solution (i.e., 27.2 g KH₂PO₄ + 1.35 ml H₃PO₄ in 1 liter water with 1:1 acetonitrile) (Uslu et al. 2008). The excavated sand was then washed three times with the same amount of extraction solution. CIP concentrations in

the solutions were determined with HPLC. The CIP retention was then calculated for each sand section.

Modeling CIP Transport in Saturated Porous Media

One dimensional advection-dispersion equation coupled with reaction terms was used to simulate the transport. See Chapter 2 modeling section.

Results and Discussion

CIP Species in Solution and Characteristics of Sand

The charges carried by CIP's functional groups are important as they impact its interactions with Cu, Ca and sand. CIP can exist in three forms (cationic, zwitterionic, and anionic forms) in DI water with two active function groups including carboxyl and piperazinyl groups. At pH 5.6, 100% of piperazinyl groups existed as $-\text{NH}_2^+$ and 20% of carboxyl groups presented as $-\text{COO}^-$ (Chapter 3, Table 3-1). In comparison, >99% Cu and Ca were present as free cations based on Visual-MINTEQ (data not shown). When coexisting with metal cations in solution, CIP can complex with Cu or Ca via its carboxyl and keto groups to form metal-CIP or metal-CIP₂ complexes. In this study, since the amounts of Cu or Ca (1 mM) used were much greater than CIP (0.7 μM), only 1:1 metal-CIP was important (Li et al. 1994; Turel et al. 1996). It should be noted that CIP-cation complexation reactions in solution were reversible, especially when cations with low complexation ability (e.g., Ca) compete with cations with strong ability (e.g., $\equiv\text{Fe}/\text{Al}$) (Turel et al. 1996; Aristilde and Sposito 2008).

In this experiment, the net surface charge for clean sand at pH 5.6 was negative with its PZC (point of zero charge) being 5.0-5.1 (Chapter 3, Table 3-3), with the majority of negative sites being from broken edges of silicon oxides ($\equiv\text{SiO}^-$), which sorb

CIP via electrostatic attraction of its piperazinyl groups. Different from clean sand, native sand was coated with small amount of Fe/Al oxides (167 and 1,087 mg/kg Fe and Al) (See Chapter 3 Table 3-3). The PZC for Fe/Al oxides is 10 (Essington 2003) so locally the broken edges of Fe/Al oxides carried positive charges ($\equiv\text{FeOH}^+$ and $\equiv\text{AlOH}^+$). The broken edges of Fe/Al oxides with high energy are highly reactive and can complex with CIP, forming surface complexation (Yost et al. 1990; Molis et al. 2000; Duckworth and Martin 2001) (Figure 4-1C). In addition, Fe/Al oxides can also sorb Cu/Ca ($\equiv\text{Fe/AlO}-\text{Ca}^{2+}$ or $\equiv\text{Fe/AlO}-\text{Cu}^{2+}$) (Figure 4-1C). Trivedi et al. (2001) demonstrated that Cu as a transition metal is more likely to bind to high affinity site on goethite whereas Ca as an alkaline earth metal bind to low affinity site (Trivedi et al. 2001; Violante et al. 2003).

CIP Transport in Saturated Sand Porous Media

The breakthrough curve (BTC) of CIP transport in clean sand column in DI water (i.e., pH = 5.6 and IS = 0 mM) was significantly delayed compared to that of bromide, the conservative tracer (Figure 4-2A). After applied to the clean sand column, CIP was first detected in the effluents ~30 PV. The BTC then slowly climbed to a peak value at ~60 PV and stayed there during further CIP injection (Figure 4-2B). Compared to the bromide BTC in the column, the delayed CIP transport indicated significant CIP retardation in sand media, which was consistent with the result in Chapter 2. The normalized peak concentration (C/C_0) of CIP was ~70%. The breakthrough experiment demonstrated that CIP interacted with the clean sand. This interaction was attributed to the electrostatic attraction between positively-charged CIP and negatively-charged clean sand ($\equiv\text{SiO}-\text{CIP}^+$) (Taboada-Serrano et al. 2005; Huang et al. 2012) (Figure 4-1B). At pH of 5.6, CIP carried both positively-charged piperazine group (100% NH_2^+ ,

Table 3-1) and negatively-charged carboxyl group (20% $-\text{COO}^-$, Table 3-1). Our data were consistent with the literature where many studies have observed the low mobility of CIP in soils (Stoob et al. 2007; Unold et al. 2009).

Simulations of the transport model matched well with the BTC of CIP transport in clean sand with $R^2 = 0.99$ (Figure 4-1). The best-fit retardation factor R of CIP in the clean sand column was 21.7 (Table 4-1). The best-fit kinetic reaction rate k of CIP was 0.018 min^{-1} , suggesting part of the CIP was retained in the sand column through kinetic reactions.

Different from CIP transport in clean sand, its transport in native sand column showed no breakthrough response under DI water condition (data not shown). No CIP was detected in the effluents even after prolonged injection as much as 100 PV. This indicated strong retardation/deposition of CIP onto native sand. In native sand, in addition to surface charge from clean sand, it was also coated with Fe/Al oxides. Besides the electrostatic attraction between CIP and sand, the retention of CIP onto the sand column was primarily attributed to strong complexation between CIP and Fe/Al oxides (Riley et al. 1993; Turel et al. 1994) (Chapter 3 Figure 3-3). The $\log k$ of complexation constant of $\text{Fe}(\text{CIP})_3$ and $\text{Al}(\text{CIP})_3$ was 46.9 and 43.6 (Turel et al. 1996). The fact that no CIP came out from native sand indicated that the complexation of CIP with $\equiv\text{Fe}/\text{Al}$ on the sand surface was much stronger than sorption of CIP by $\equiv\text{SiO}^-$.

The strong CIP retention in native sand column was consistent with the literature examining its sorption behaviors in soils (Nowara et al. 1997; Gu and Karthikeyan 2005; Carrasquillo et al. 2008; MacKay and Seremet 2008). As discussed above, CIP sorption onto sand in DI water systems was mainly controlled by two mechanisms: 1) weak

electrostatic attraction between CIP functional groups and heterogeneously-charged sand surfaces (Hari et al. 2005), and 2) strong complexation between CIP carboxyl groups and Fe/Al oxides on sand surface (Nowara et al. 1997) (Figure 4-1C). These two mechanisms were confirmed in the literature through batch and spectroscopic studies of CIP sorption onto Fe/Al oxides and aluminosilicates (Nowara et al. 1997; Goyne et al. 2005; Gu and Karthikeyan 2005; Oker and Akmehmet-Balcloglu 2005).

Presence of Cu and Ca promoted CIP transport in Clean Sand

When CIP was mixed with Cu^{2+} and Ca^{2+} , it existed primarily as Cu-CIP^+ and Ca-CIP^+ , with little free CIP in solution (Figure 4-1A). In addition, excess amounts of Cu^{2+} and Ca^{2+} in comparison to Cu-CIP^+ and Ca-CIP^+ were also present in the solution. Compared to CIP alone, presence of Cu or Ca significantly promoted CIP transport in clean sand (Figure 4-3). The BTC of CIP in the presence of Ca or Cu was significantly advanced. The BTC of CIP transport in the clean sand column in presence of Cu (i.e., pH = 5.6, CIP = 0.7 μM and Cu = 1 mM) was only slightly retarded than the BTC of bromide, the conservative tracer (Figure 4-2A). After applied to the sand column, CIP was detected in the effluents ~1.5 PV. The BTC then quickly climbed to a peak value at ~4 PV and stayed there during further injection (Figure 4-3A). Compared to the CIP transport in clean sand under DI water condition (30 PV), the BTC of CIP in the presence of Cu showed significant advance, indicating much reduced attraction between CIP and sand surface. Similar to Cu, the presence of Ca also significantly increased CIP transport in clean sand (Figure 4-3B). CIP was detected in the effluents ~1.5 PV, then quickly climbed and reached peak value at ~5 PV. The normalized peak concentration (C/C_0) of CIP was ~ 0.7 (Figure 4-3B), which was 25% less than that in presence of Cu (C/C_0 ~ 0.95) (Figure 4-3A), indicating that Ca was less effective in

mobilizing CIP from clean sand than Cu. This was consistent with the fact that clean sand had greater sorption capacity of Cu than Ca (2.99 vs. 0.03 mg/kg; Chapter 3 Table 3-3).

At pH 5.6, 100% of piperazinyl groups of CIP existed as NH_2^+ and 20% of carboxyl groups presented as COO^- (Chapter 3 Table 3-1). When CIP was transported in clean sand, it was effectively sorbed onto negatively charged sand surface ($\equiv\text{SiO}^-$; Figure 4-1B). However, when CIP was mixed with Cu or Ca, the sand was ineffective in sorbing CIP as it existed as Cu-CIP^+ or Ca-CIP^+ . This was probably because aqueous Cu^{2+} and Ca^{2+} outcompeted Cu-CIP^+ or Ca-CIP^+ to be sorbed onto sand surface, leaving more CIP in the solution.

This hypothesis was supported by batch experiment where 1 mM of Cu or Ca was mixed with 0.7 μM CIP (Table 4-2). The ability of clean sand in sorbing CIP was reduced from 0.5 to 0.03 mg kg^{-1} in the presence of Cu and from 0.5 to 0.38 mg kg^{-1} in the presence of Ca (Table 4-2). In this aspect, clean sand was more effective in sorbing Cu than Ca, with CIP being the least effective among the three (Figure 4-1B).

Though Cu/Ca effectively increased CIP transport, the BTC of CIP in the presence of either Ca or Cu showed some delay, indicating slight retardation of CIP in the porous media. This was probably due to the attraction of CuCIP^+ or CaCIP^+ onto negatively charged sand surface.

Simulations of the transport model matched well with the BTCs of CIP in the presence of Ca or Cu, with R^2 of 0.93 and 0.97, respectively (Table 4-1). The best-fit retardation factor R of CIP in the column was 1.71 (Ca) and 1.17 (Cu). The best-fit kinetic reaction rate k of CIP in the column was 0.016 min^{-1} (Ca) and 0.0031 min^{-1} (Cu),

compared to bromide tracer with 0 min^{-1} , suggesting some of the CIP was retained in the sand column through kinetic reactions with more in the presence of Cu than Ca. The modeling results further confirmed that Cu was more effective than Ca in increasing CIP mobility in clean sand.

Presence of Cu Promoted CIP Transport in Native Sand

In the presence of 1 mM Cu, CIP was detected in the effluents ~30 PV after applied to the native sand column. The BTC then slowly climbed to a peak value and stayed there during further CIP injection. The normalized peak concentration (C/C_0) of CIP was ~ 1 after 60 PV compared to ~0.7 after 5 PV in clean sand. Clearly, compared to clean sand, it was much easier for Cu-CIP⁺ to be sorbed onto native sand. In native sand, besides quartz sand, Fe/Al oxides were also present. With stronger complexation of CIP to Fe/Al than Cu (Gu and Karthikeyan 2005), Fe/Al oxides on native sand surface enhanced CIP sorption onto sand (Figure 4-1C). Since native sand had stronger sorption capacity for CIP than clean sand (1.4 vs. 0.5 mg/kg; Table 4- 3), it took much longer for CIP to come out from clean sand than native sand (60 vs. 5 PV).

Unlike Cu, when Ca was present in the solution, CIP could not be detected even at 100 PV, indicating much weaker complexation of CIP with Ca. The log k of Ca-CIP⁺ and Cu-CIP⁺ complexation constant was 11.3 and 14.7 (Turel et al. 1996), so Cu-CIP⁺ was 3-order magnitude stronger than Ca-CIP⁺. As a result, it was easier for CIP to disassociate from Ca-CIP⁺, leaving CIP to be sorbed onto sand via strong complexation with Fe/Al on the sand surface. This was supported by the batch data where 1.7 and 1.0 mg/kg CIP was sorbed onto native sand in the presence of Ca and Cu (Table 4-2). Our result indicated that the presence of metals in aqueous phase and surface

chemistry of porous media were both important in controlling CIP retention and transport in sand media.

Aqueous Cu and Ca Increased Transport of Presorbed CIP in Clean Sand

In the previous experiment, CIP was mixed with Cu or Ca. In this experiment, CIP was presorbed onto sand and then leached with aqueous Cu or Ca. Both Cu and Ca mobilized CIP presorbed on sand surface, with Cu being more effective than Ca (Figure 4-4). After Cu was applied to sand column presorbed with CIP, CIP was detected around 1.5 PV, then quickly climbed to peak around 2 PV. The normalized peak concentration (C/C_0) of CIP was ~ 1.8 . This number was greater than unity because presorbed CIP onto sand column was released in short period when Cu was eluted in the column. Mass balance calculations showed that 90% of CIP was remobilized from sand column. The results demonstrated that Cu was effective in mobilizing CIP presorbed in clean sand. This could be attributed to the fact that the interaction between CIP and clean sand was mostly via electrostatic attraction. When significant amount Cu (1 mM) solution flushed the column, Cu^{2+} effectively replaced the CIP^+ on the sand surface since they were both positively charged (Figure 4-1B). This was similar to the experiment where Cu^{2+} was mixed with CIP, with Cu being more effective being sorbed onto clean sand surface than Cu-CIP⁺.

Similar to Cu, after Ca was applied to sand column with presorbed CIP, CIP was detected ~ 1.5 PV, then quickly climbed to peak ~ 3 PV. The normalized peak concentration (C/C_0) of CIP was ~ 0.25 . Mass balance calculations showed that 30% of CIP was remobilized from sand column compared to 90% by Cu, reflecting Ca was less effective in desorbing CIP from sand surface. This was consistent with the fact that clean sand was more effective in sorbing Cu than Ca (Table 4-2).

Aqueous Cu and Ca didn't Enhance Transport of Presorbed CIP in Native Sand

Since no CIP was detected after 100 PV of DI water, 1 mM Ca or 1 mM Cu, a separate profile study was conducted. Analysis of the retained CIP profile in the sand column after 5 PV of DI water flushing showed that almost all (~95%) CIP was retained in the bottom 1-cm layer of the column and the rest of 5% of CIP being in the second 1-cm layer from the bottom (Figure 4-5), suggesting strong interactions between CIP and the native sand grains (Figure 4-1C).

Similar to DI water, analysis of the retained CIP profile in the sand column after flushing with Ca solution showed that 94% the CIP was retained in the bottom 1-cm layer and the rest of 6% of CIP being found in the second 1-cm layer from the bottom (Figure 4-5). This suggested the limited effect of Ca on mobilizing the CIP presorbed on the native sand surface. Unlike Ca, analysis of the retained CIP profile in the sand column after 5 PV of 1 mM Cu solution flushing showed that 88%, 10%, and 2% CIP was retained in the first, second, third 1-cm layer from the bottom, respectively (Figure 4-5).

The results were consistent with the hypothesis that sorption of CIP onto native sand via complexation with $\equiv\text{Fe}/\text{Al}$ was stronger and more effective than that via electron static attraction onto $\equiv\text{SiO}^-$. Once sorbed onto $\equiv\text{Fe}/\text{Al}$ surface, Ca or Cu was probably unable to replace CIP. However, CIP sorbed onto $\equiv\text{SiO}^-$ surface can be replaced by Ca (~1%) or Cu (~7%). So more likely, those mobilized CIP was from $\equiv\text{SiO}^-$ surface. Though limited in quantity, $\equiv\text{Fe}/\text{Al}$ was more effective sorbing CIP than $\equiv\text{SiO}^-$ as supported by the batch data where 1.4 mg/kg CIP was sorbed by native sand compared to 0.5 mg/kg by clean sand (Table 4-2).

Conclusions

CIP can interact with metals in aqueous and solid phase, which has not been studied in details. This study showed that while surface metal oxides was effective in immobilizing CIP in soils, solution phase Ca and Cu facilitated CIP transport. Critical factors in facilitating or impeding CIP transport included: 1) the type of metal cation, which controls the stability of the complex formed; 2) the concentration of metals present; and 3) whether the cations are in aqueous or solid phase.

Although only aqueous Cu and Ca were investigated in present study, CIP could form complexes with majority of metal cations in soils (Turel 2002), suggesting other metals could have similar effect on CIP transport. In addition, almost all FQs share the same carboxyl, keto and piperazine reactive functional group like CIP, similar chemodynamics process was expected for other FQs. Therefore, other major cations in aqueous and soil environment should also be evaluated to better understand the chemodynamics of FQs in soils and receiving waters, which is critical for comprehensive assessment of the potential environmental risk of FQs.

Table 4-1. Transport model parameters and experiment conditions for CIP transport in sand columns.

| No. | Sand | Influent | R | k | R ² |
|-----|--------|----------|-------|--------|----------------|
| 1 | Clean | DI water | 21.7 | 0.018 | 0.99 |
| 2 | Native | DI water | ---- | ---- | ---- |
| 3 | Clean | 1 mM Ca | 1.71 | 0.016 | 0.93 |
| 4 | Native | 1 mM Ca | ----- | ---- | ---- |
| 5 | Clean | 1 mM Cu | 1.17 | 0.0031 | 0.97 |
| 6 | Native | 1 mM Cu | 22.0 | 0.0054 | 0.99 |

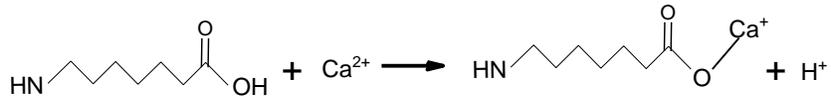
The influent consisted of 0.7 μM CIP with DI water, 1 mM Ca or 1 mM Cu.

When Fe/Al oxides from native sand were removed, they were referred to as clean sand R is the retardation factor, which reflects the magnitude of equilibrium reactions in the sand column; k is the kinetic reaction rate constant (min^{-1}) and D is the dispersion coefficient ($\text{cm}^2 \text{min}^{-1}$).

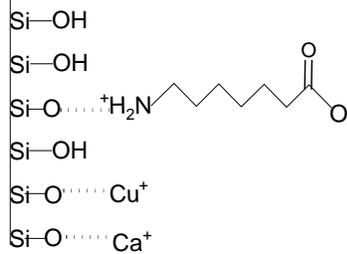
Table 4-2. Partition coefficient of CIP onto clean and native sand after shaking 3 g of sand with 0.7 μM CIP plus water, 1 mM Ca or 1 mM Cu for 24 h.

| Treatment | Cu/Ca sorbed on sand (mg kg^{-1}) | | CIP sorbed on sand (mg kg^{-1}) | |
|-----------|---|-----------------------|---|----------------|
| | Clean | Native | Clean | Native |
| DI water | under detection limit | under detection limit | 0.5 \pm 0.06 | 1.4 \pm 0.01 |
| 1 mM Cu | 2.99 \pm 0.02 | 210.40 | 0.03 \pm 0.00 | 1.0 \pm 0.01 |
| 1 mM Ca | 0.03 \pm 0.03 | 0.21 \pm 0.06 | 0.38 \pm 0.09 | 1.7 \pm 0.11 |

A) CIP forms stronger complex with Cu than Ca in solution



B) Electrostatic attraction of CIP, Ca and Cu onto clean sand surface



C) Complex of CIP with Fe/Al and electrostatic attraction of Ca and Cu onto Fe/Al oxides on native sand surface

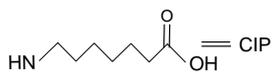
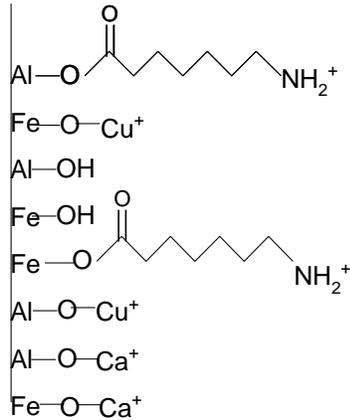


Figure 4-1. Possible interactions of CIP with clean and native sand.

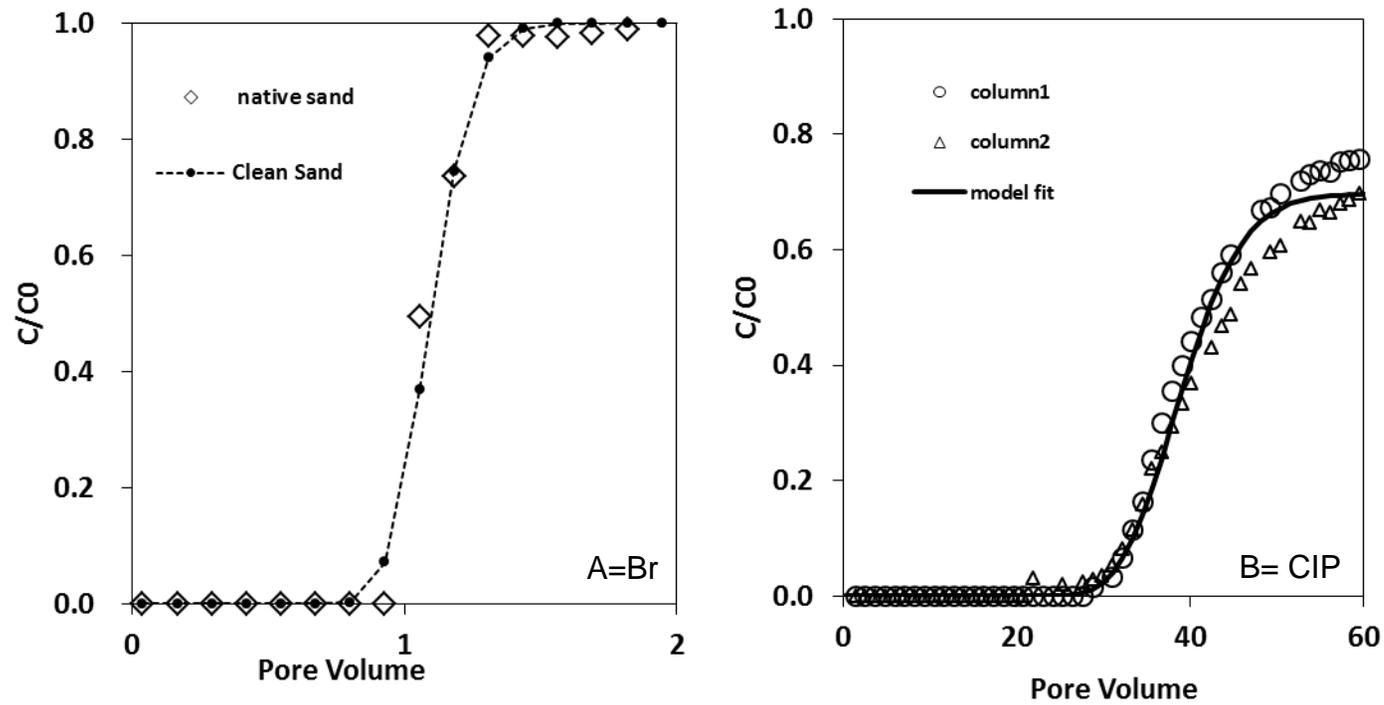


Figure 4-2. Transport of bromide in native and clean sand and CIP in clean sand media under DI water condition. When Fe/Al oxides from native sand were removed, they were referred to as clean sand

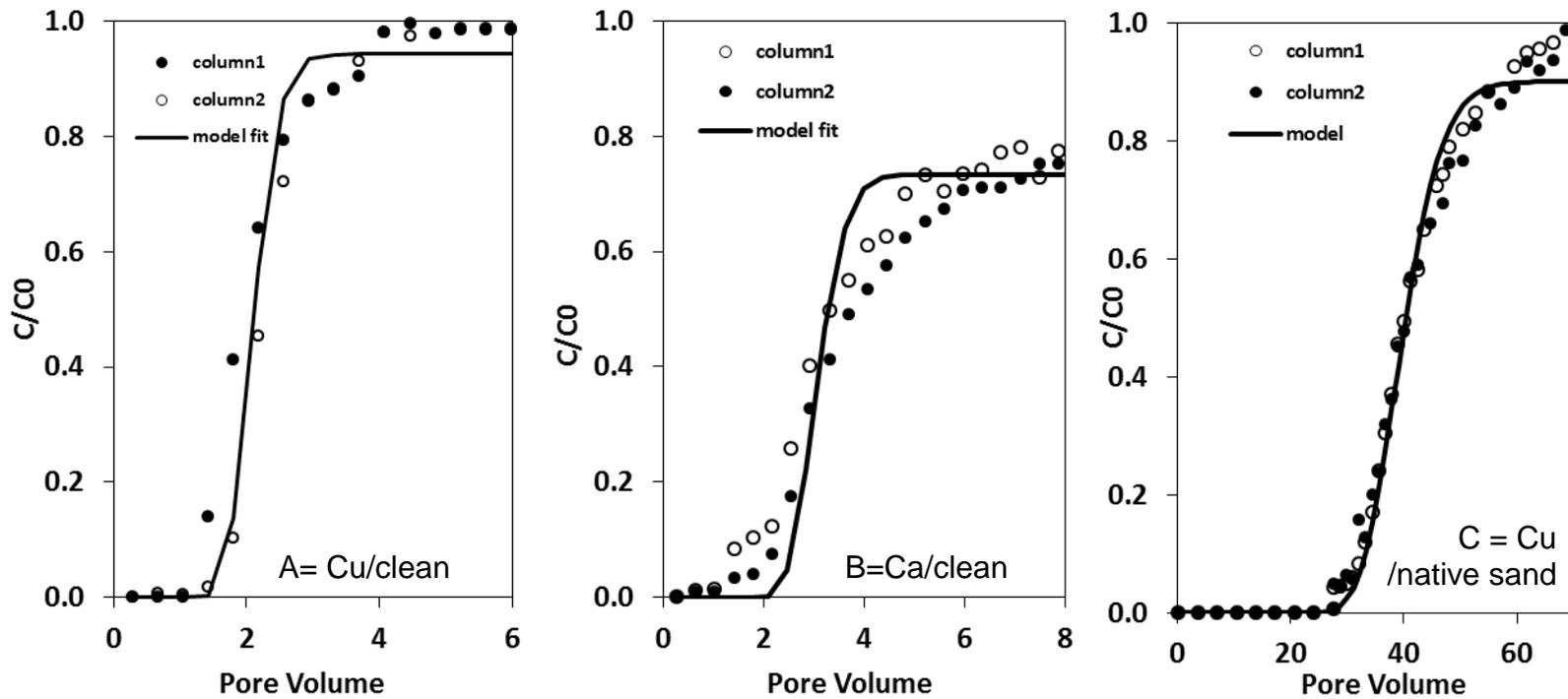


Figure 4-3. CIP transport in saturated sand media where it was mixed with: 1 mM Cu in clean sand, 1 mM Ca in clean sand and Cu in native sand. When Fe/Al oxides from native sand were removed, they were referred to as clean sand.

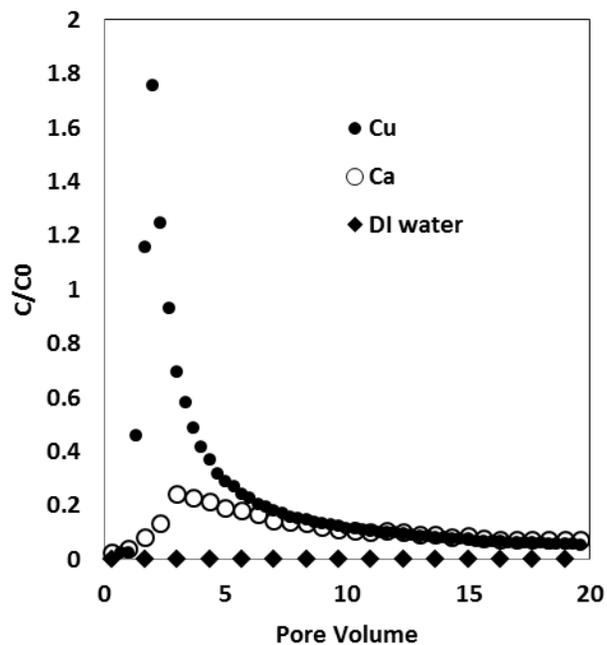


Figure 4-4. Mobilization of presorbed CIP onto sand after column was flushed with DI water, 1 mM Ca, and 1 mM Cu for 5 pore volumes

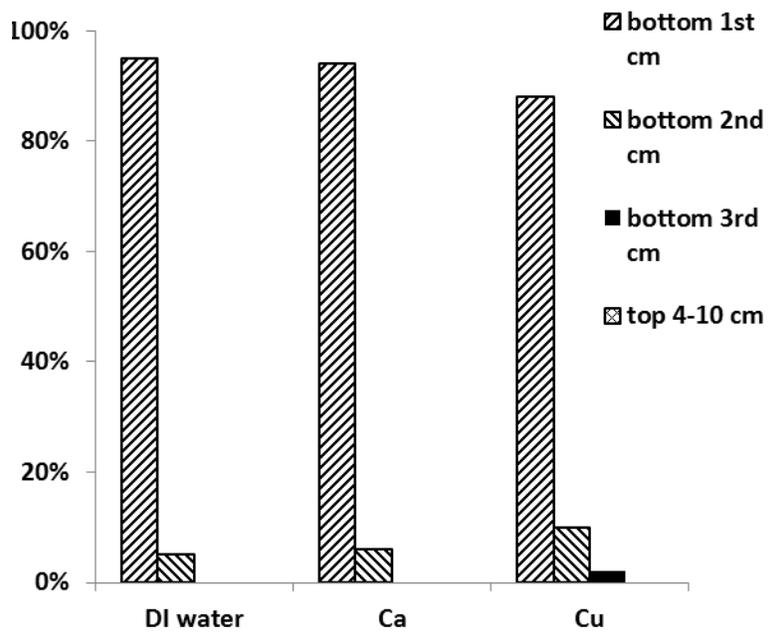


Figure 4-5. Distribution of sorbed CIP in different layers of the saturated native sand column after flushed with DI water, 1 mM Ca or 1 mM Cu.

CHAPTER 5 COLLOID FACILITATED CIP TRANSPORT IN SATURATED POROUS MEDIA

Introduction

Small particles in subsurface have been characterized as mobile sorbent can facilitate contaminants transport. Colloids, defined as soil particles with diameters less than 10 micrometers (Essington 2003), are widely recognized as mobile particles in the subsurface. These colloids have gained growing concern due to their high affinity to contaminants and risks to human health (Roy and Dzombak 1997).

Many organic contaminants, generally considered to be highly retarded due to strong interactions with immobile aquifer material, also have a high affinity for the mobile colloidal material. Consequently, the association between the contaminant and colloid ultimately affects the transport of the contaminant (Roy and Dzombak 1997). The mobility potential predicted by batch adsorption experiment may be misleading in consideration of the presence of colloids as mobile sorbents (Grolimund and Borkovec 2005). The significance of colloidal facilitated transport depends on the following factors: (a) the identity and concentration of colloids; (b) the nature of the interaction between the contaminants and the colloids; and (c) the mobility of the colloids in an aquifer.

Although it is known that mobile colloids can enhance the mobility of contaminants, there are not many experimental studies focusing on colloid-facilitated contaminant transport in water-saturated porous media Sen and Khilar (2006). After entering water-saturated media, most of the chemical contaminants and natural colloids interact with each other and with the surrounding media. These interactions may alter the dynamic behavior of contaminants, and increase the difficulty to understand the fate and transport of contaminants in the media.

Inorganic colloids include clay, metal oxides, and inorganic precipitates in the sub micrometer size range (Essington 2003). These colloids occur both naturally and from anthropogenic sources. These colloids were detected in the ground water down gradient from the disposal site indicating that the colloids were mobile in the aquifer system.

Ciprofloxacin (CIP) is one of the most widely prescribed fluoroquinolone antibiotics, also the main metabolite of enrofloxacin. The sources of CIP in the environment include land application of sewage sludge, wastewater irrigation, and disposing of expired pharmaceutical prescriptions (Golet et al. 2002), making CIP of increasingly environmental concern. With highly reactive functional group, CIP has been demonstrated can highly associated with certain natural colloids like Fe/Mn oxides (Gu and Karthikeyan 2005), geophite and montmorillonite (Carrasquillo et al. 2008). In particular, no research has yet been published on the influence of natural clay on CIP mobility in saturated porous media. Little information is currently available concerning the association between inorganic colloids and organic contaminants. Due to both the existing data base concerning organic contaminant sorption to typical subsurface mineral surfaces and to inorganic colloidal transport, the scientific framework suggests facilitated transport is a viable transport mechanism.

It is therefore crucial to examine the enhanced CIP transport in water-saturated porous media. This study was designed to improve the current understanding of colloid-facilitated organic contaminant transport in saturated porous media. Clay colloids and CIP were used in laboratory experiments to examine their transport and cotransport behaviors in water-saturated sand columns. Our objectives were 1) to compare the

transport of kaolinite, montmorillonite and CIP in water-saturated porous media; 2) to examine the colloid facilitated CIP transport in saturated porous media; 3) to examine the effects of kaolinite and montmorillonite on the mobility of CIP in saturated porous media.

Materials and Methods

Materials

Ciprofloxacin (ACS 85721-33-1) was purchased from Applichem (Darmstadt, Germany). Its chemical structure and basic information are presented in Chapter 3.

Quartz sand treatment and basic information was same as chapter 3.

Kaolinite and montmorillonite powders (EM Science, Gibbstown, N.J.) were used to make colloids according to the procedures reported by Gao et al. 2004. The mean sizes of the colloids, as determined by photon correlation spectroscopy, did not vary significantly during the experiments and equaled 0.80 μm for kaolinite and 0.65 μm for montmorillonite.

Batch Adsorption Experiments

Adsorption experiments of CIP to kaolinite and montmorillonite were conducted using polytetrafluoroethylene centrifuge tubes as completely mixed batch vessels. Each vessel was filled with a predetermined amount of sorbent about 500 ppm kaolinite or 200 montmorillonite and 50-mL target CIP solutions 0, 0.5, 1, 1.5, 2, 5, 10ppm. The vessels were shaken for more than 24 h (predetermined) to reach apparent sorption equilibrium, and then centrifuged to separate the solids from the liquid phase. Aliquots of supernatant were withdrawn to determine liquid phase CIP concentrations using HPLC (Waters 2695, Milford, MA) equipped with a fluorescence detector (Waters 2475, Milford, MA).

Column Experiments

Column packing method was same as Chapter 2 (Modeling Antibiotic Transport in Saturated Porous Media section). Bromide was applied to the column as a conservative tracer for the breakthrough studies, see chapter 2, Column Experiments section.

CIP Colloid Co-transport in Sand Media

Four types of colloid suspensions were used: pure kaolinite, kaolinite-CIP suspension, pure montmorillonite and montmorillitnite -CIP suspension. The CIP-colloid suspension was applied to the column as a 2 pore volume pulse, and then the column was flushed with DI water for 4 pore volume. Colloid concentrations were determined by measuring the total extinction of light with UV-visible spectrophotometry wavelength 350 nm for Kaolinite and 275nm for montmorillitnite. The CIP concentration was determined by HPLC. The experiments were conducted in duplicates.

Colloids Mobilization of CIP Presorbed onto Sand Media

In the previous experiment, CIP and colloids were transported simultaneously in the sand column. To determine the ability of colloids in mobilizing CIP presorbed in sand media, CIP was applied to the column at a 2 PV pulse, and then the column was flushed with 500 ppm Kaolinite or 200 ppm montmorillitnite. Experiment was terminated when out flow stabilized or after 6 PV. Effluent samples were collected from the top of the column with a fraction collector during flushing with kaolinite or montmorillitnite solution after CIP injection pulse to analyze CIP concentrations. All experiments were performed in duplicate.

Results and Discussion

CIP Sorption Isotherm onto Sand, Kaolinite and Montmorillonite

Both Kaolinite and montmorillonite were able to sorb CIP, but their ability differed substantially. The sorption of CIP onto native sand was significantly higher than clean sand. CIP sorption data fit well with Langmuir equation ($R^2 = 0.998-0.999$; data not show). The best-fit value of maximum CIP sorption capacity from Langmuir model were 60mg kg^{-1} for Kaolinite and 30 g kg^{-1} for montmorillonite, CIP sorbed to montmorillonite significantly higher than Kaolinite.

Montmorillonite possesses 95% permanent charge, 5% pH dependent charge, and a higher CEC, than kaolinite, which possesses 95% pH dependent charge, 5% permanent charge, and lower CEC (Essington 2003) . Previous research suggested the CEC is one of the key factors for CIP sorption to soil minerals (Carrasquillo et al. 2008; Vasudevan et al. 2009). At pH of 5.6, amine groups ($-\text{NH}^{2+}$) of CIP was ~100% positively charged, hence, high CIP sorption onto montmorillonite can be attributed to columbic attraction between positively-charged CIP amine groups ($-\text{NH}^{2+}$) and negatively-charged site in montmorillonite. To better understand the mechanism of CIP sorption to montmorillonite, one experiment was conducted to exam the sodium released form montmorillonite while CIP was sorbed. The result showed sodium released is directly linear proportional to CIP sorbed to montmorillonite further confirmed cation exchanged happened when CIP sorbed.

Although lack of CEC (0.28 cmol kg^{-1}), native sand also showed relatively high sorption ability to CIP. This was mainly attributed to Fe/Al oxides on the surface (Fe/AlO^+), which amounted to 167 and 1,087 mg kg^{-1} (Table 3-4, chapter 3). Previous study indicated surface complexation of Fe/Al oxides with CIP was with its carboxylic

acid group (Gu and Karthikeyan 2005). The method using both native sand and clean sand (chapter3) also confirmed the surface complexation process happened on native sand surface.

CIP, Kaolinite and Montmorillonite Transport in Saturated Sand Column

Transport of kaolinite and montmorillonite was performed in saturated sand column. Based on the breakthrough curve of montmorillonite and kaolinite were both same to that of conservative tracer bromide, indicating that no interaction between these two colloids and sand media (Fig. 5-1). This could be attributed to the fact that the interaction between colloids and porous medium were repulsive because both surfaces were negatively charged under experimental conditions. The zeta potential of the quartz sand used in this experiment was -19.7 mV, confirming it was negatively charged.

Different from colloids, CIP show no breakthrough curve, see chapter 2, CIP transport part.

CIP Colloid Co-transport in Sand Media

Kaolinite has similar breakthrough curve like bromide tracer, CIP have no effect on kaolinite transport (Fig. 5-3). However, there was no break though for CIP co-transport with kaolinite, indicating much weaker complexation of CIP with kaolinite. As a result, it was easier for CIP to disassociate from CIP-Kaolinite complexion, leaving CIP to be sorbed onto sand via strong complexation with Fe/Al on the sand surface.

In the presence of montmorillonite, CIP was detected in the effluents about 1 PV after applied to the native sand column. The BTC then quickly climbed to a peak value and stayed there during further CIP-montmorillonite injection(Fig. 5-2). The CIP concentrations decreased quickly to zero when the columns were flushed with CIP-free

solution. The normalized peak concentration (C/C_0) of CIP was ~ 1 . Exactly same breakthrough curve was detected for montmorillonite, the correlation between CIP and montmorillonite concentrations revealed a strong linear relationship with R^2 of 0.99 (data not shown). The linear relationship can be used to simplify the models by assuming that the CIP and montmorillonite are always directly proportional during the transport process. The high ability of montmorillonite as CIP carrier could be explained by the high sorption ability to CIP of montmorillonite. Also previous research demonstrated CIP could be sorbed to montmorillonite interlayer (Wang et al. 2011). The interaction between sand and CIP sorbed between the montmorillonite layers can be limited since directly.

Transport of Presorbed CIP in Native Sand under the Influence of Colloids

In the previous experiment, CIP was mixed with colloid. In this experiment, CIP was presorbed onto sand and then leached with colloids suspension. After CIP presorbed into sand, both Kaolinite and montmorillonite have similar breakthrough curve to colloid transport in DI water. That indicate although small amount CIP was adsorbed to the sand may alter the surface character of sand, it is not significant enough to change the interaction between colloids and sand.

Kaolinite could not mobilize CIP presorbed on sand surface, no CIP could be detected in the effluence while flushing column with kaolinite suspension (Figure 5-4). However, montmorillonite was much more effective to mobilize CIP. After montmorillonite was applied to sand column presorbed with CIP, CIP was detected around 0.8 PV, then quickly climbed to peak around 2 PV. The normalized peak concentration (C/C_0) of CIP was ~ 1.6 (Figure 5-5). This number was greater than unity because presorbed CIP onto sand column was released in short period when montmorillonite was eluted in the column. Mass balance calculations showed that 99%

of CIP was remobilized from sand column. The results demonstrated that montmorillonite was effective in mobilizing CIP presorbed in sand. The higher affinity of montmorillonite for CIP over sand resulted in CIP to be stripped from the sand and sorbed onto the montmorillonite. When montmorillonite solution flushed the column, CIP was detached to sand surface and relocated to montmorillonite particles. The much higher mobilization ability of montmorillonite to CIP presorbed in sand column, indicating the sorption ability of colloids was the key factor to control the mobilization capacity.

Environmental Implication

In this experiment only montmorillonite was tested, other colloids like goethite also has been reported have high sorption ability, as a result they could also be effective to facilitate CIP transport. Besides, soil minerals like iron or aluminum oxides have also been demonstrated to have strong sorption to CIP (Gu and Karthikeyan 2005), they could also perform as CIP carrier for CIP transport. Therefore the conclusion that CIP is low mobility chemical in soil system based on the strong CIP sorption to soil minerals is misleading. In consider of high concentration of CIP in water body has been reported colloid facilitated CIP transport should not be overlooked.

Conclusions

I examined the effects of colloid kaolinite and montmorillonite on CIP transport in saturate porous media. Our results indicated that: 1) montmorillonite has higher sorption ability to CIP than Kaolinite; 2) premixed montmorillonite can facilitate CIP transport, Premixed Kaolinite could not facilitated CIP transport; 3)montmorillonite can remobilize presorbed CIP, kaolinite cannot remobilize presorbed CIP. Our research demonstrated the importance of colloids in controlling the fate and transport of CIP in the environment.

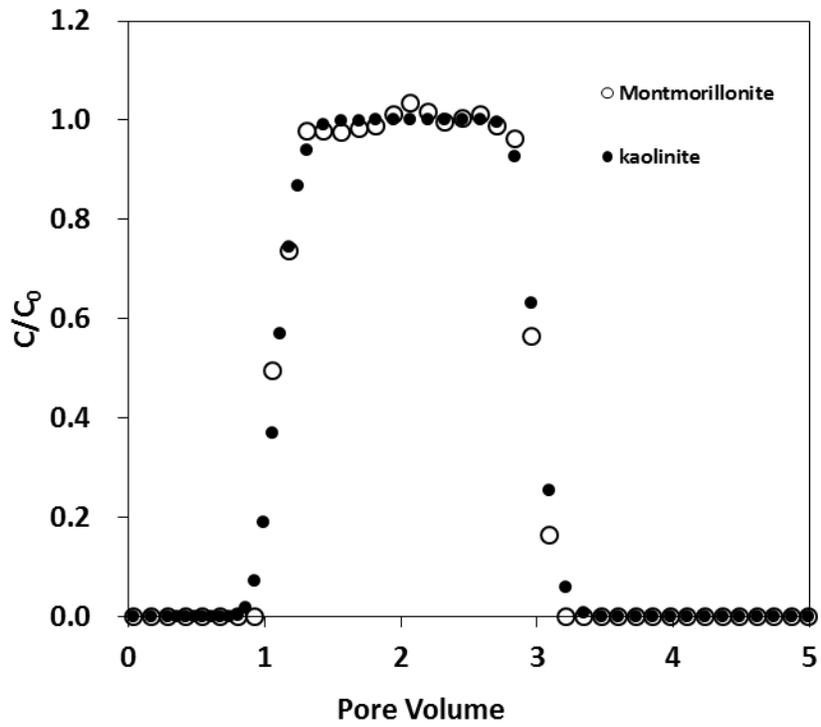


Figure 5-1. Transport of kaolinite and montmorillonite in sand media under DI water.

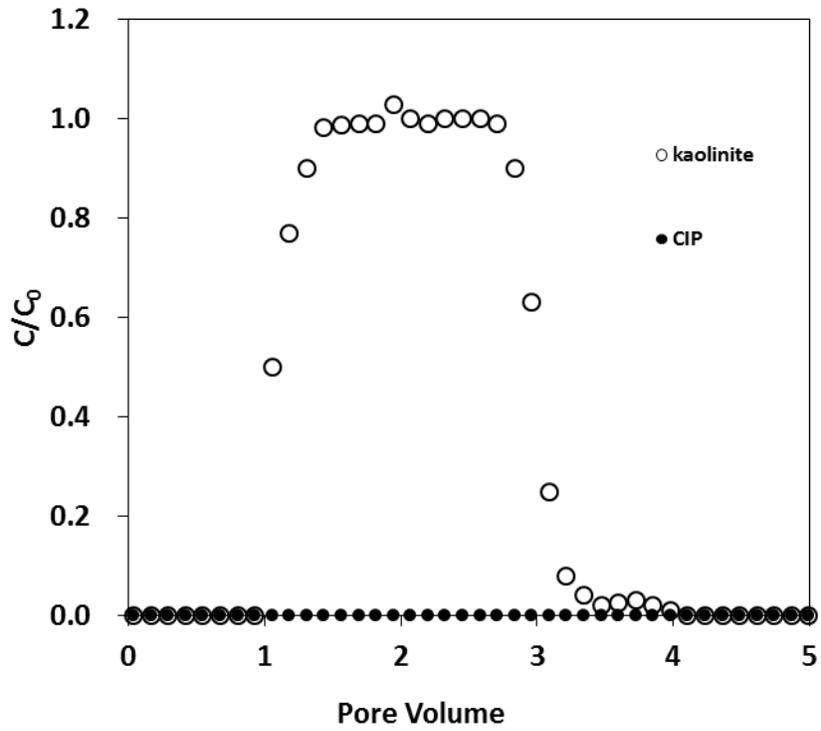


Figure 5-2. Premixed CIP and kaolinite co-transport in saturated sand media.

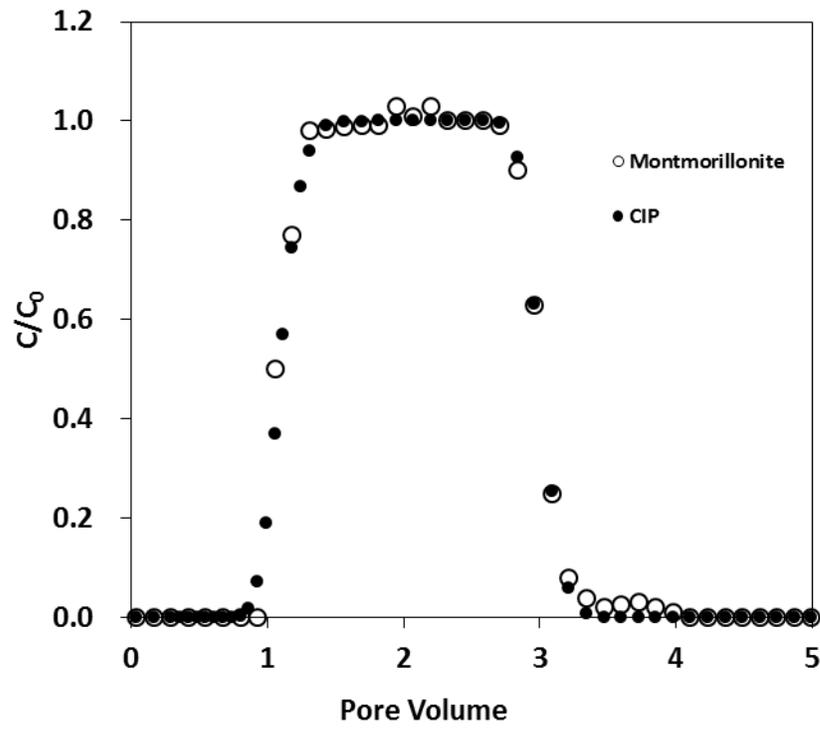


Figure 5-3. Premixed CIP and montmorillonite co-transport in saturated sand media.

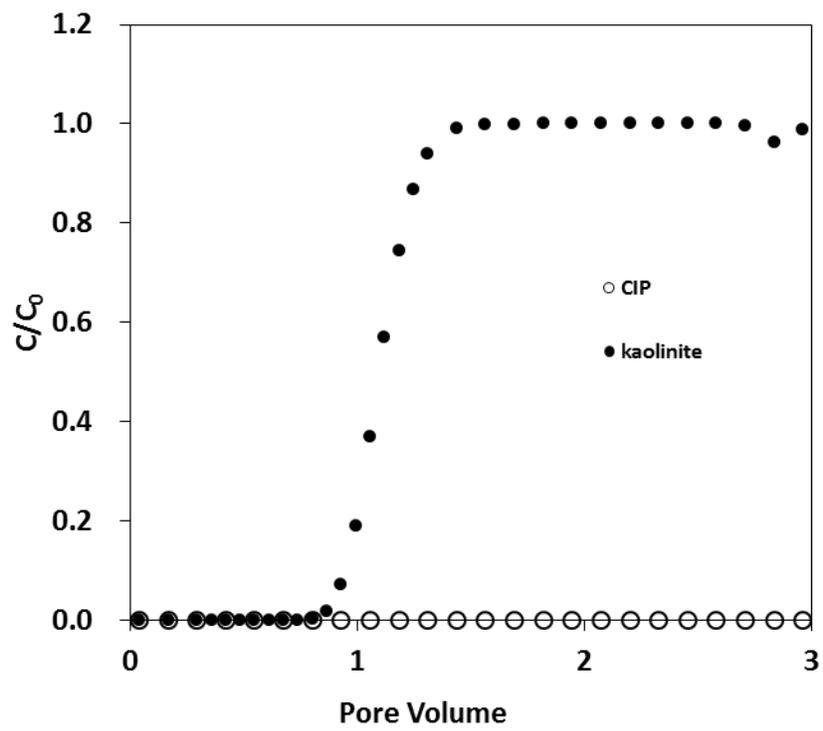


Figure 5-4. Mobilization of presorbed CIP onto sand after column was flushed with montmorillonite.

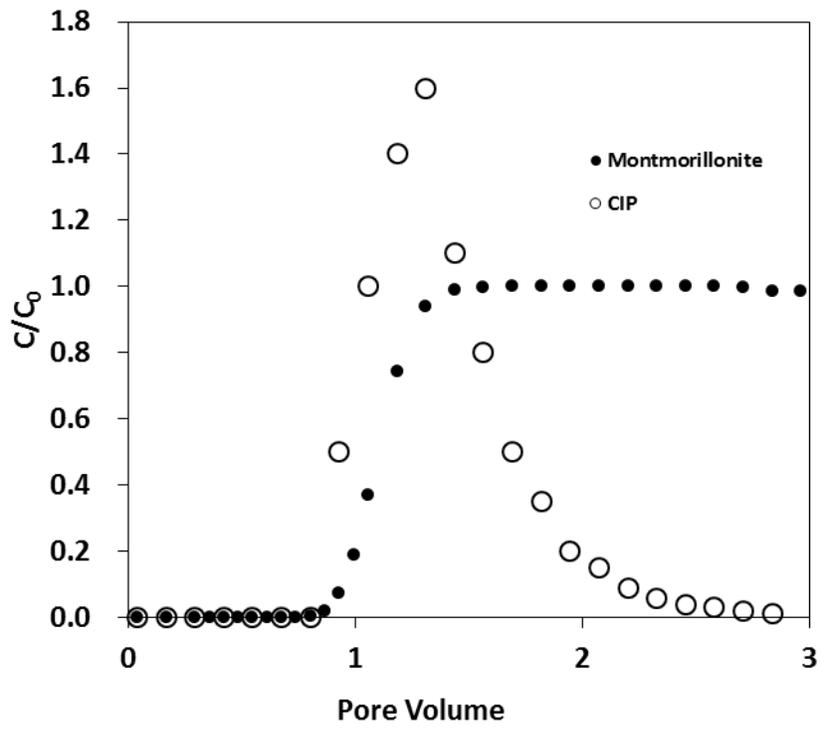


Figure 5-5. Mobilization of presorbed CIP onto sand after column was flushed with Kaolinite.

CHAPTER 6 CONCLUSIONS AND FUTURE DIRECTIONS

Laboratory batch and column experiments were conducted to examine the effects of solution chemistry (i.e., ionic strength (IS) and pH) on the retention and transport of two antibiotics in saturated porous media. Our results indicated that: 1) sulfamethoxazole (SMZ) was more mobile in saturated porous media than ciprofloxacin (CIP); 2) Solution pH played an important role in controlling the transport of CIP, but showed little effect on the transport of SMZ under the experimental conditions tested; 3) Solution IS had little effects on SMZ transport under the two pH conditions (9.5 and 5.6) but slightly enhanced the mobility of the retained CIP in the sand column at pH 5.6; and 4) traditional solute transport model could be used to simulate the retention and transport of antibiotics in water-saturated porous media.

In addition to solution IS and pH, CIP can interact with metals in aqueous and solid phase, which has not been studied in details. Our study showed that while surface metal oxides was effective in immobilizing CIP in soils, solution phase Ca and Cu increase the mobility of CIP. Our results indicated that: 1) Fe/Al oxides on native sand surface was responsible for CIP sorption; 2) Fe/Al oxides played an important role in cation bridging effect of Cu and Ca; 3) both Cu and Ca decreased CIP sorption onto clean sand; and 4) both Cu and Ca promoted CIP sorption onto native sand, but at higher concentrations, they decreased CIP sorption. Our research demonstrated the importance of cations such as Cu and Ca in controlling the fate and transport of CIP in the environment.

Column experiments were designed, in consider of the primary disadvantage of the batch technique for measuring retardation factor is that it does not necessarily

reproduce the chemical reaction conditions that take place in the real environment. To better understand the chemodynamic behaviors of CIP in soils and receiving waters, column experiment was designed to investigate critical factors in facilitating CIP transport or remobilize CIP presorbed in the sand column. These critical factors included: 1) cation with stronger complexation ability with CIP have better ability to facilitate CIP transport ; 2) cations with stronger complexation ability also more effectively remobilize presorbed CIP; 3) cations can not effectively mobilized CIP presorbed to native sand.

CIP has been reported with great sorption ability in batch experiment in different soils, indicating low mobility. However, widely present divalent cations like Cu or Ca could significantly influence CIP behavior in CIP sorption and transport. For CIP sorption batch research using Ca to adjust ionic strength (Zhang and Dong 2008; Wang et al. 2009), it may not be appropriate to precisely reflect the equilibrium CIP sorption behavior. For the dynamic transport system, Ca also has significant ability to promote CIP transport. In respect of the widely exist Ca in soil system, this promoted mobility of CIP should not be overlooked.

Although only aqueous Cu and Ca were investigated in present study, CIP could form complexes with majority of metal cations in soils (Turel 2002), suggesting other metals could have similar effect on CIP transport. Some cations like Fe with extremely high complexation ability can be equivalent to much higher concentration of low complexation ability cations like Ca (Turel et al. 1996). To better understand the total effect of all the cations high concentration cations were also used, since the effect of these metals can add up. In addition, almost all FQs share the same carboxyl, keto and

piperazine reactive functional group like CIP, similar chemodynamics process was expected for other FQs. Therefore, other major cations in aqueous and soil environment should also be evaluated to better understand the chemodynamics of FQs in soils and receiving waters, which is critical for comprehensive assessment of the potential environmental risk of FQs.

Colloids with strong sorption ability to CIP can also facilitate CIP transport. In this experiment only montmorillonite was tested, other colloids like goethite also has been reported have high sorption ability, as a result they could also be effective to facilitate CIP transport. Besides, soil minerals like iron or aluminum oxides have also been demonstrated to have strong sorption to CIP (Gu and Karthikeyan 2005), they could also perform as CIP carrier for CIP transport. Therefore the conclusion that CIP is low mobility chemical in soil system based on the strong CIP sorption to soil minerals is misleading. In consider of high concentration of CIP in water body has been reported colloid facilitated CIP transport should not be overlooked.

To conclude, the chemical property of antibiotics greatly influence on their fate and transport in the environment. Although SMZ and CIP were investigated in present study, similar process was expected for other antibiotics with similar functional group. Therefore, other major solution chemistry properties in aqueous and soil environment should also be evaluated to better understand the fate and transport of these antibiotics in soils and receiving waters, which is critical for comprehensive assessment of the potential environmental risk of antibiotics.

APPENDIX A
ADSORPTION ISOTHERMS OF CIP TO SAND : A-1) NATIVE SAND, A-2) CLEAN SAND

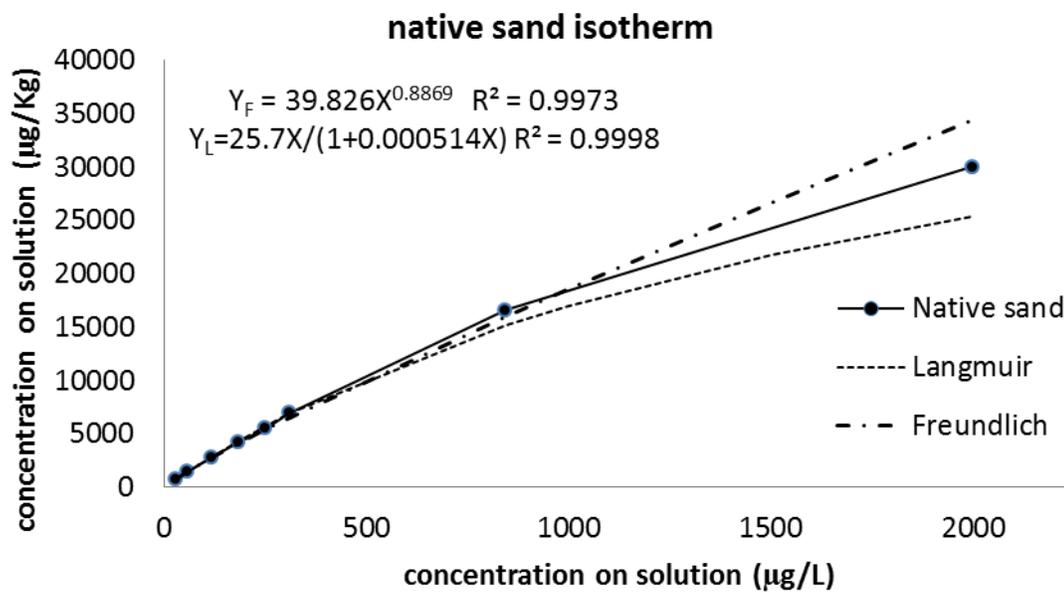


Figure A-1. Native sand isotherm CIP sorption isotherm

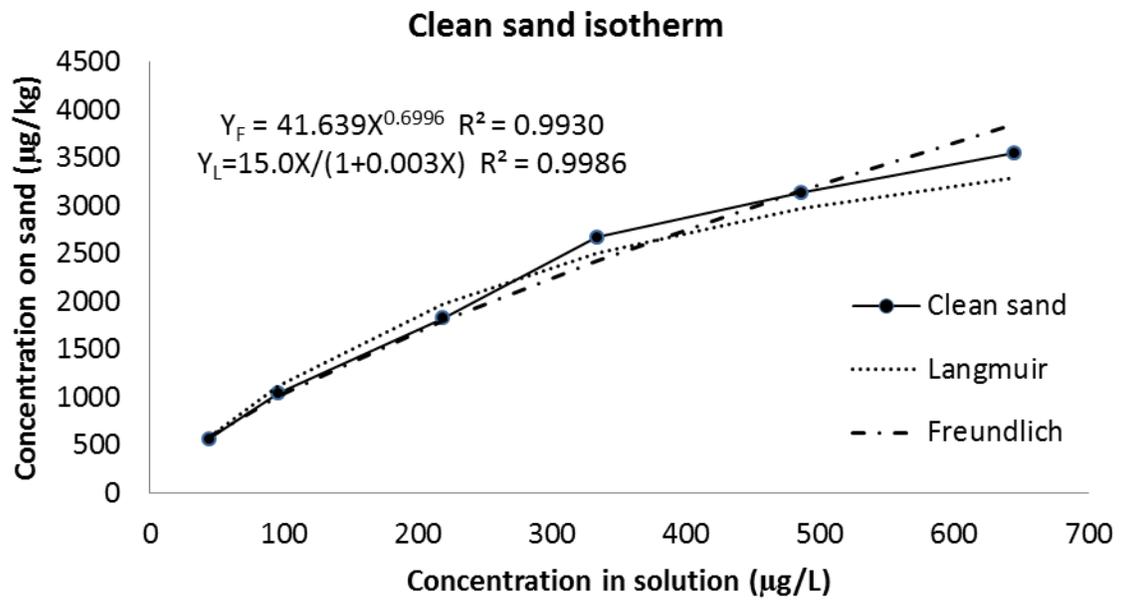


Figure A-2. Clean sand isotherm CIP sorption isotherm

APPENDIX B
SCANNING ELECTRON MICROSCOPE IMAGE OF NATIVE SAND

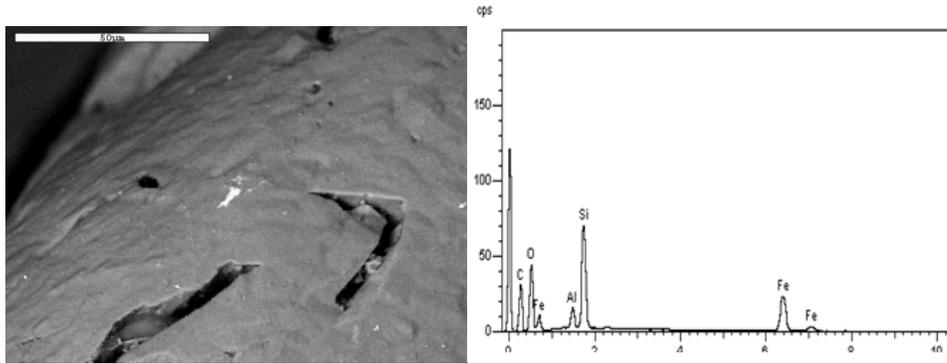


Figure B-1 Scanning electron microscope Image of native sand

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BIOGRAPHICAL SKETCH

Hao Chen, the only child of her parents, was born and brought up in Yan Cheng city, Jiangsu province, China. After graduated from high school, she joined the Nanjing University of Technology for her bachelor's degree in environmental engineering in 2004. Following this, she obtained a scholarship for pursuing a master's degree in environmental science at the Institution of Soil Science, Chinese Academy of Sciences. Her master's thesis was titled "Sulfonamides antibiotic residual in Jiangsu province and sorption on Taihu paddy soil". In 2008, she joined the University of Florida, Gainesville, Florida to pursue her PhD in Soil and Water Science Department. Here she was a graduate research assistant and worked on the antibiotic sorption and transport in porous media.