Adsorption of Sulfonamide Antimicrobial Agents to Clay Minerals

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Adsorption of three sulfonamide antimicrobials to clay minerals was investigated as a function of pH, ionic strength, and type of exchangeable cation. Sulfonamide antimicrobial adsorption exhibited pronounced pH dependence consistent with sorbate speciation and clay properties. Sulfonamide antimicrobials did not intercalate into montmorillonite, and surface charge density influenced sorption by determining adsorption domain size. Adsorption edge data were best fit to a model including terms for the cationic and uncharged species. Adsorption of uncharged sulfamethazine to montmorillonite was relatively insensitive to pH, ionic strength, and type of exchangeable cation, while that to kaolinite was highly sensitive to ionic strength. Adsorption of cationic sulfamethazine to montmorillonite exceeded that of the neutral species by 1–2 orders of magnitude, but was unimportant for kaolinite at the pH values examined. Cation exchange appeared to contribute to sorption of cationic sulfonamide species to montmorillonite. Anionic sulfamethazine adsorption was negligible. The nature of the sulfonamide R group influenced the degree of adsorption of cationic and neutral species. Our results highlight the importance of considering sulfonamide speciation and clay surface charge density in predicting the transport of these antimicrobials.

Introduction

Sulfonamide antimicrobials comprise a class of synthetic, primarily bacteriostatic, sulfaminamide derivatives and find use in human therapy, livestock production, and aquaculture. Treated individuals excrete a fraction of the administered dose as the unaltered parent compound or an acetylated deacetylation (1, 2) susceptible to reactivation by bacterial (3). Sulfonamides enter the environment through disposal of domestic and hospital waste and via runoff and infiltration from confined animal feeding operations and fields treated with animal manure. Although sulfonamide antimicrobials are susceptible to biodegradation by sewage sludge microorganisms (4, 5), hydraulic residence times in biological treatment processes are often insufficient for their elimination from treated effluent (5). Sulfonamide antimicrobials ranked among the most frequently detected pharmaceuticals in U.S. streams (6) and have been found in groundwater (7), landfill leachate (8), and runoff from and soils beneath effluent-irrigated lands (9, 10).

The primary concern with introducing antimicrobial agents into soil and water environments is the spread of antibiotic resistance in response to increased selective pressure, potentially leading to proliferation of resistant pathogens. Schmitt et al. (11) reported that exposure of soil microcosms to 7.3 mg·kg⁻¹ sulfachloropyridazine resulted in increased bacterial community tolerance to this sulfonamide. Because sulfonamide antimicrobials may be present in groundwater and surface water resources used as drinking water supplies, and can cause allergic reactions in sensitive individuals (12), human exposure to these compounds may also warrant concern.

Understanding sulfonamide antimicrobial sorption to soil and sediment particles is essential for assessing their potential to leach into groundwater and to be transported in aquifers and surface waters. Because sulfonamides must enter bacterial cells to interfere with folate metabolism, sorption to environmental particles impacts the selective pressure exerted by these compounds. Previous studies of sulfonamide sorption focused on whole and size-fractionated soils, rather than specific well-defined sorbent phases (13–15). Sulfonamides sorbed weakly to bulk soil, with greater sorption to fine silt and clay particle-size fractions (13). Thiele-Bruhn et al. (13) emphasized the importance of natural organic matter in sulfonamide sorption to soils. To date, clay mineral constituents of the soil clay fraction have not been examined as sorbents for sulfonamides, although smectites are often important sorbents for polar and ionizable organic compounds, including antimicrobial agents from other classes (16–22).

The objectives of this study were to investigate the adsorption of three sulfonamide antimicrobial agents to common phyllosilicate minerals and to derive sorption parameters applicable over a range of solution conditions. Reference montmorillonite and kaolinite clays were selected so that we could examine sulfonamide antimicrobial interaction with these important soil minerals without interference from organic matter coatings. We obtained clay adsorption data under a range of solution conditions by separately varying proton activity, ionic strength (I), and type of exchangeable cation.

Materials and Methods

Chemicals. The chemicals used, suppliers, and purities are described in the Supporting Information (SI). Tables 1 and S1 present structures and physicochemical properties of the sulfonamide antimicrobials studied. Sulfonamide antimicrobials possess two ionizable functional groups considered relevant to the environmental pH range: the anilinic amine and the amide moieties. The cationic species (SA⁺) dominates at low pH values; the neutral form (SA⁰) is the principal species at pH values between pK₁ and pK₃; and the anionic species (SA⁻) is the main form at higher pH values (Figure S1). A minor zwitterionic species (SA⁺⁻) is in tautomeric equilibrium with SA⁻ (contribution to overall speciation <0.2%) (23). Tautomeric constants (Kᵣ = [SA⁺⁻]/[SA⁺]) for sulfamethazine (SMZ) and sulfapyridine (SPY) are 10⁻⁸.³⁰ and 10⁻¹² (23).

Sorbents. Reference montmorillonite (SWy-2 and SAz-1) and kaolinite (KGa-1b) clays were obtained from the Clay Minerals Society Source Clays Repository (West Lafayette, IN). Clay preparation, homoionization, permanent charge reduction, and characterization are described in the SI. Table 2 presents characteristics of the clay minerals.

Batch Sorption Experiments. We conducted sorption edge experiments to investigate the pH-dependent adsorp-
tion of SMZ, sulfamethoxazole (SMX), and SPY to near homoionic clays. Isotherm experiments were conducted to examine SMZ adsorption as a function of sorbate concentration. We refer to the clays in these experiments as near homoionic because at pH values less than ~6, protons occupy some cation exchange sites. Experimental details are provided in the SI. Briefly, SMZ (3H-labeled or unlabeled), SPY (unlabeled), or SMX (unlabeled) were equilibrated with near homoionic clay at room temperature for 2.5 h in the dark. Adsorption edge experiments were conducted in 10 mM NaHCO3/Na2CO3; adsorption isotherm experiments were conducted in 10 mM acetate buffer. Phase separation was achieved by 10-min centrifugation at 9200 g. Supernatants were analyzed by liquid scintillation counting (for SMZ) or high-performance liquid chromatography with UV detection (for SMX and SPY). Desorption of SMZ from clays with a pH above 6.5 resulted in a decrease in adsorption to a minimum value at pH ~9. Between pH 5.5 and 7, experimental adsorption coefficients for Na–KGa-1b and Na–SWy-2 did not differ significantly on a sorbent mass basis (p > 0.05). Here, and throughout the paper we employ Student's t test to determine if the adsorption coefficients for Na–KGa-1b and Na–SWy-2 did not differ significantly on a sorbent mass basis (p > 0.05).

Results and Discussion

X-ray Diffraction Analysis. Neutral and cationic sulfonamide species did not intercalate into SWy-2, but interacted primarily with external surfaces (see SI). We cannot exclude that part of the molecule (e.g., the anilinium group) was able to enter the interlayer space at the edges of smectite particles. That SMZ did not intercalate into SWy-2 is not surprising given the pronounced nonplanarity of the molecule (Table S1).

Adsorption Edge Experiments. Sulfonamide antimicrobial adsorption to phyllosilicate minerals exhibited pronounced pH dependence consistent with sorbate speciation and clay properties. We examined adsorption between pH 3.5 and 9.3, a range encompassing proton activities found in most natural environments. Proton activities higher than 10^{-3.5} M were not investigated because smectite clays spontaneously decompose at low pH values (~30). Figure 1 displays SMZ adsorption edges for sodium-saturated montmorillonite and kaolinite clays. Sulfamethazine adsorption to montmorillonite clays (SWy-2 and SAz-1) exhibited only slight pH-dependence between pH 4.5 and 7. Decreases in pH below ~5 resulted in a marked increase in SMZ adsorption to Na–SAz-1 below pH ~8. As pH increased beyond this point, adsorption approached zero for both smectites.

In contrast to the smectites, SMZ adsorption to the kaolinite clay displayed no pH-dependence between pH 4.3 and 7, proton activities at which the neutral sulfamethazine species (SMZ^0) dominated solution speciation (Figure S1). The lack of pH dependence for SMZ^0 adsorption suggests that the variable charge edge sites of kaolinite did not contribute substantially to overall sorption. Increases in pH above 6.5 resulted in a decrease in adsorption to a minimum value at pH ~9. Between pH 5.5 and 7, experimental adsorption coefficients for Na–KGa-1b and Na–SWy-2 did not differ significantly on a sorbent mass basis (p > 0.05). Here, and throughout the paper we employ Student’s t test...
to determine the significance of differences between treatments.

To examine the contributions of individual sulfonamide species to overall sorption, we followed the approach of Figueroa et al. (22) to calculate species-specific equilibrium adsorption coefficients. The overall adsorption coefficient at a given pH value can be represented as the sum of the contributions of the individual species (31):

\[ K_d = K^S\text{A}_d^+ \cdot \alpha_{SA}^+ + K^S\text{A}_d^0 + K^S\text{A}_d^+ \cdot \alpha_{SA} + K^S\text{A}_d^+ \cdot \alpha_{SA} \]  

(1)

where \( K_d \) (L·kg\(^{-1}\)) is the overall equilibrium adsorption coefficient and is defined as the ratio of the sorbed and dissolved concentrations; \( K^S\text{A}_d^+ \), \( K^S\text{A}_d^0 \), and \( K^S\text{A}_d^+ \cdot \alpha_{SA} \) are the adsorption coefficients of the cationic, uncharged, zwitterionic, and anionic species, respectively; and \( \alpha_{SA}^+ \), \( \alpha_{SA} \), and \( \alpha_{SA}^+ \) represent the mass fractions of these species in bulk solution. The model formulation did not explicitly consider possible altered sulfonamide speciation at the clay surface. Experimental data were fit to eq 1 using the General Linear Model (SYSTAT 10.2, SYSTAT Software, Point Richmond, CA). We evaluated each sorption term to determine whether model fits were improved by its inclusion. Except in a minority of cases (indicated below), inclusion of the anionic species did not improve model fits to the experimental data (\( p > 0.05 \)). Desorption of sulfonamide antimicrobials from smectite surfaces at pH 10 provided additional evidence that anionic species generally did not participate in sorption interactions. In no case were model fits improved by inclusion of a term for the zwitterion (\( p > 0.05 \)). Therefore, in most cases adsorption edge data were modeled using terms for only cationic and uncharged species:

\[ K_d = K^S\text{A}_d^+ \cdot \alpha_{SA}^+ + K^S\text{A}_d^0 \]  

(2)

For SMZ adsorption to Na–SWy-2 and Na–SAz-1, the empirical model was best fit with \( K^S\text{A}_d^+ \) values of 2620 ± 250 L·kg\(^{-1}\) and 771 ± 8 L·kg\(^{-1}\) and \( K^S\text{A}_d^0 \) values of 12.0 ± 0.7 L·kg\(^{-1}\) and 2.3 ± 0.6 L·kg\(^{-1}\) (Table 3). For Na–Kga-1b, the best fit to the empirical model was obtained using a \( K^S\text{A}_d^+ \) value of 14.8 ± 0.6 L·kg\(^{-1}\); inclusion of \( K^S\text{A}_d^0 \) did not improve the model fit (\( p > 0.05 \)).

The empirical model results allowed evaluation of the relative contributions of SMZ species to overall adsorption. Between pH 2.3 and 7.4, SMZ\(^0\) was the main solution species. Nevertheless, the model fit to the experimental data indicated that cationic sulfamethazine (SMZ\(^+\)) dominated adsorption to Na–SWy-2 up to pH 4.6 (a proton activity at which SMZ\(^+\) comprised only 0.43% of solution species). Between pH 5 and 7, SMZ\(^0\) dominated solution speciation (99–72%) and contributed the most to overall adsorption based on the model-derived sorption parameters (72–99%). Over this pH range adsorption to Na–SWy-2 exhibited relatively little pH dependence. As pH increased above 7.4, the anionic species (SMZ\(^-\)) assumed dominance in solution, and the adsorption coefficient approached a minimum value, consistent with increasing electrostatic repulsion of SMZ\(^-\) by the negatively charged smectite surface as proton activity declined. Sorption parameters obtained in this study are strictly valid for only the buffer system employed. However, distribution coefficients from experiments using unbuffered, bicarbonate-buffered, and acetate-buffered solutions did not differ significantly (\( p > 0.05 \); data not shown).

Consideration of the nature of the clay surfaces and SMZ solution speciation allows rationalization of the observed trends in adsorption with pH. Due to isomorphic substitution of primarily Mg\(^2+\) for Al\(^3+\) in the dioctahedral layer (32) and variable charge edge sites, the smectites carry net negative charge over the pH range examined (33), are cation exchangers, and exhibit negative adsorption of anions (34). Electrostatic forces favor SMZ\(^+\) association with the siloxane surface and disfavor adsorption of the anion. At higher proton activities, SMZ adsorption to Na–SWy-2 did not parallel changes in bulk solution speciation. Instead, SMZ\(^+\) dominated adsorption ~2.5 pH units above that expected from bulk solution speciation. This effect may be attributed to enhanced dissociation of water solvating the exchange ions on the smectite surface (35) and the increasing replacement of exchangeable Na\(^+\) by protons as pH decreases below ~6. Kaolinite carries a net negative charge at pH values above 3.5 (36). However, due to the low charge density and lower pH\(_{\text{HMC}}\) of kaolinite, SMZ\(^-\) adsorption was unimportant at the pH values examined. Dissociation of water at kaolinite surfaces is not significantly enhanced over that of bulk water due to the very low density of exchange sites.

Because sulfonamides appeared to interact with external smectite surfaces, we normalized modeled species-specific adsorption coefficients by the clay BET surface areas (Table 3). On a surface area basis, SMZ\(^0\) adsorption to Na–SWy-2 exceeded that to Na–SAz-1 by a factor of 15.2 ± 1.4. The surface-area-normalized \( K^S\text{A}_d^+ \) value for Na–SWy-2 was larger than that for Na–SAz-1 by a factor of 9.9 ± 1.0. Over the pH range at which SMZ\(^0\) dominated solution speciation, surface-area-normalized experimental \( K_d \) values were higher for Na–Kga-1b than for the montmorillonites (\( p < 0.05 \)).

Effect of Surface Charge Density. Montmorillonite surface charge density appeared to influence SMZ sorption. On a mass basis, SAz-1 surface charge as measured by CEC was considerably higher than that of SWy-2 (Table 2). Sulfamethazine sorption to the low-charge SWy-2 substantially exceeded that to the high-charge SAz-1 at proton activities at which SMZ\(^0\) dominated solution speciation, especially below pH ~5 (Figure 1). On the basis of the fit of the experimental data to eq 2, SMZ\(^0\) and SMZ\(^-\) adsorption to Na–SWy-2 was larger than that to Na–SAz-1 by factors of 5.2 ± 1.4 and 3.4 ± 0.3 on a sorbent mass basis (Table 3). Normalization of \( K^S\text{A}_d^+ \) by the CEC of the external clay

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**TABLE 3. Distribution Coefficients for SMZ Adsorption to Near Homoionic Clays**

<table>
<thead>
<tr>
<th>sorbent</th>
<th>( K^S\text{A}_d^+ ) (L·kg(^{-1}))</th>
<th>( K^S\text{A}_d^0 ) (L·kg(^{-1}))</th>
<th>( R^2 )</th>
<th>( K^S\text{A}<em>d^+ /A</em>{\text{extsurf}} ) (L·m(^{-3})·mol(^{-1}))</th>
<th>( K^S\text{A}<em>d^0 /A</em>{\text{extsurf}} ) (L·m(^{-3})·mol(^{-1}))</th>
<th>( K^S\text{A}<em>d^2 /CEC</em>{\text{extsurf}} ) (L·mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li–SWy-2</td>
<td>3240 ± 460</td>
<td>15.4 ± 0.8</td>
<td>0.97</td>
<td>109 ± 16</td>
<td>0.52 ± 0.03</td>
<td>108 ± 16</td>
</tr>
<tr>
<td>Na–SWy-2</td>
<td>2620 ± 250</td>
<td>12.0 ± 0.7</td>
<td>0.96</td>
<td>82.4 ± 7.9</td>
<td>0.38 ± 0.02</td>
<td>81 ± 9</td>
</tr>
<tr>
<td>Mg–SWy-2</td>
<td>b</td>
<td>17.1 ± 0.8</td>
<td>0.95</td>
<td>b</td>
<td>0.66 ± 0.03</td>
<td>b</td>
</tr>
<tr>
<td>K–SWy-2</td>
<td>1070 ± 44</td>
<td>16.2 ± 0.8</td>
<td>0.99</td>
<td>44.0 ± 1.8</td>
<td>0.67 ± 0.03</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>Ca–SWy-2</td>
<td>728 ± 88</td>
<td>22.2 ± 0.6</td>
<td>0.98</td>
<td>26.7 ± 2.2</td>
<td>0.81 ± 0.02</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Na–SAz-1</td>
<td>771 ± 8</td>
<td>2.3 ± 0.6</td>
<td>&lt;0.001</td>
<td>8.3 ± 0.1</td>
<td>0.02 ± 0.01</td>
<td>7.7 ± 0.2</td>
</tr>
<tr>
<td>Na–Kga-1b</td>
<td>b</td>
<td>14.8 ± 0.6</td>
<td>0.97</td>
<td>b</td>
<td>1.26 ± 0.05</td>
<td>b</td>
</tr>
</tbody>
</table>

* Ionic strength = 10 mM; values represent best fits of experimental data to eq 2 (mean ± standard error). Abbreviations: \( A_{\text{extsurf}} \), external surface area (cf. Table 2); CEC_{\text{extsurf}}, cation exchange capacity of external surfaces (see Table S2). b Model fit not improved by including a term for the cation.
surfaces indicated that SMZ\(^{+}\) adsorption to Na—SWy-2 surpassed that to Na—SAz-1 by a factor of 11 ± 1. Using measured CEC values (Table 2), a surface area per unit cell (excluding edge surfaces) of 0.93 nm\(^2\)/unit cell, and the layer silicate molecular mass of 746.21 g mol\(^{-1}\) (37), and assuming regular spacing of negative charges on siloxane surfaces, average distances between adjacent sodium ions on external surfaces were calculated to be 0.90 nm for Na—SWy-2 and 0.67 nm for Na—SAz-1. The average adsorption domain area of the low-charge SWy-2 exceeded that of the high-charge SAz-1 and apparently influenced the degree of SMZ sorption. This argument is not free from ambiguity because isomorphic substitution by Al\(^{3+}\) for Si\(^{4+}\) in the tetrahedral layer of SWy-2 but not in SAz-1 (32) and more extensive ordering of charge deficit sites in the octahedral sheet of SWy-2 (38) may have contributed to differences in SMZ adsorption.

To eliminate the ambiguity associated with comparing smectites differing in tetrahedral charge and ordering of charge deficit sites and to further investigate the influence of surface charge density on SMZ adsorption, we conducted sorption edge experiments with charge-reduced SAz-1 using heat-treated Na—SAz-1 as a control. In charge-reduced clays, heat treatment caused Li\(^{+}\) to migrate into the smectite octahedral sheet and compensate part of the permanent negative charge (39). This treatment permanently reduces surface charge density, spreads the remaining excess negative charge over a larger number of surface oxygen atoms, and increases the size of adsorption domains between exchangeable cations (35, 40). Model fits to adsorption edge data indicated that charge reduction significantly enhanced adsorption of SMZ\(^+\) and SMZ\(^-\) to SAz-1 (p < 0.05) compared to the heat-treated control sample (Table 4). Mixing charge-reduced SAz-1 at a 0.7:0.3 Na-to-Li ratio (22.7% reduction in charge density) increased \(K_{d}^{SMZ^{+}}\) by a factor of 4.3 ± 0.4 and \(K_{d}^{SMZ^{-}}\) by a factor of 1.9 ± 0.5; mixing at a 0.3:0.7 Na-to-Li ratio (79.5% reduction in charge density) resulted in \(K_{d}^{SMZ^{+}}\) and \(K_{d}^{SMZ^{-}}\) increasing by factors of 4.6 ± 0.4 and 3.8 ± 0.7.

These results demonstrate the sensitivity of SMZ adsorption to reduction in clay surface charge density; the size of available adsorption domains on the siloxane surface (i.e., areas unoccupied by exchangeable cations) was apparently important for sulfonamide sorption. On the basis of the measured CEC values for the charge-reduced clays (Table 2) and the assumptions used above, the average spacing between adjacent Na\(^{+}\) atoms on external surfaces of Na—SAz-1 and charge-reduced clays were 0.70 nm for heat-treated Na—SAz-1, 0.87 nm for 0.7Na:0.3Li—SAz-1, and 2.21 nm for 0.3Na:0.7Li—SAz-1. Reduction in the number of exchangeable cations and concomitant increase in sorption domain size enhanced SMZ adsorption. This phenomenon has been observed for other polar sorbates (40, 41). The ability of SMZ\(^+\) to displace exchangeable cations (see below) appears to have resulted in the availability of larger adsorption domains, enhancing adsorption of the cationic species over that of SMZ\(^0\). Charge reduction also increased the overall Lewis base softness of the siloxane surface (35), increasing the ease with which Na\(^+\) (a hard Lewis acid) can be replaced and presumably enhancing the stability of complexes with the SMZ anilinium cation (a soft Lewis acid).

### Ionic Strength Effects

Solution ionic strength influences the double layer thickness of clays and the aqueous activity of sulfonamide species. We probed ionic strength effects on SMZ adsorption to Na—SWy-2 using three salt concentrations (10 mM NaHCO\(_3\); 10 mM NaHCO\(_3\)+100 mM NaCl, and 10 mM NaHCO\(_3\)+300 mM NaCl) (Figure 2). Species-specific \(K_{d}\) values were calculated at each ionic strength by fitting experimental data to eq 2 (Table 5) applying the Davies equation (37) to correct sulfonamide speciation for ionic strength in solutions with \(I = 110 \text{mM}\) and \(310 \text{mM}\). For Na—SWy-2, little change in sorption was apparent between pH~5.5 and ~7. Below pH 5.5, experimental \(K_{d}\) values were significantly lower at ionic strength of 310 mM than those at \(10 \text{mM}\) (p < 0.05); significant differences in \(K_{d}\) values between ionic strengths of 10 mM and 110 mM became apparent at pH values below ~5 (p < 0.05). The large decreases in \(K_{d}^{SMZ^{+}}\) observed as ionic strength increased from 10 to 310 mM suggests that cation exchange contributes to SMZ\(^+\) interaction with the smectite surface. At pH values > 8.3, no SMZ sorption to Na—SWy-2 was apparent at 10 mM ionic strength, while some sorption occurred at higher ionic strength (p < 0.05). The adsorption coefficient of SMZ\(^-\) increased with ionic strength (p < 0.05) as expected from double layer compression and reduction of anion repulsion. For the higher ionic strength values, model fits to the experimental data were improved by including a term for SMZ\(^-\) sorption (p < 0.05):

\[
K_{d} = K_{d}^{0} \cdot \alpha_{SAz} + K_{d}^{SMZ^{+}} \cdot \alpha_{SMZ^{+}} + K_{d}^{SMZ^{-}} \cdot \alpha_{SMZ^{-}}.
\] (3)

The \(K_{d}^{SMZ^{-}}\) values obtained from the empirical model fits are valid for only the ionic strength values at which they were determined. To facilitate extension of these results to

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### Tables

#### Table 4. Distribution Coefficients for SMZ Adsorption to Charge-Reduced Smectite

<table>
<thead>
<tr>
<th>sorbent</th>
<th>(K_{d}^{SMZ^{+}}) (L kg(^{-1}))</th>
<th>(K_{d}^{SMZ^{-}}) (L kg(^{-1}))</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na—SAz-1</td>
<td>771 ± 8</td>
<td>2.3 ± 0.6</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>heat-treated Na—SAz-1</td>
<td>1120 ± 190</td>
<td>6.8 ± 0.5</td>
<td>0.93</td>
</tr>
<tr>
<td>charge-reduced SAz-1</td>
<td>0.3 Na: 0.7 Li—SAz-1</td>
<td>2110 ± 380</td>
<td>29.4 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>0.7 Na: 0.3 Li—SAz-1</td>
<td>4270 ± 300</td>
<td>31.4 ± 1.1</td>
</tr>
</tbody>
</table>

* Values represent best fits of experimental data to eq 2 (mean ± standard error).

#### Table 5. Effect of Ionic Strength on SMZ Adsorption to Na—SWy-2

<table>
<thead>
<tr>
<th>(I) (mM)</th>
<th>(K_{d}^{SMZ^{+}}) (L kg(^{-1}))</th>
<th>(K_{d}^{SMZ^{-}}) (L kg(^{-1}))</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2620 ± 250</td>
<td>12.0 ± 0.7</td>
<td>0.96</td>
</tr>
<tr>
<td>110</td>
<td>1380 ± 150</td>
<td>13.2 ± 0.2</td>
<td>0.99</td>
</tr>
<tr>
<td>310</td>
<td>750 ± 64</td>
<td>10.3 ± 0.3</td>
<td>0.96</td>
</tr>
</tbody>
</table>

* Values represent best fits of experimental data to eq 2 or 3 (mean ± standard error).
other ionic strength conditions, we calculated a stoichiometric selectivity coefficient for SMZ in the presence of competing Na\(^+\) (\(K_{\text{SMZ-}\text{Na}}\)) using the following expression (22, 42):

\[
\log(K'_{\text{SMZ}}/\text{CEC}_{\text{surf}}) = -\log(\text{Na}^+) + \log K_{\text{SMZ-}\text{Na}}
\]

where \(K_{\text{SMZ-}\text{Na}}\) carries units of equiv/ equiv. Linear regression of our data using this expression yielded a slope of -0.35 and a \(K_{\text{SMZ-}\text{Na}}\) value of 190 equiv/equiv (\(r^2 = 0.96\)). We note that our slope deviated from that expected (viz. -1). The change in \(K'_{\text{SMZ}}\) observed as ionic strength increased was less than that predicted by eq 4.

Ionic strength affected SMZ adsorption to Na-KGa-1b in a different manner (Figure S2). At \(I = 110\) mM, distribution coefficients became independent of pH and did not differ significantly from zero. We were therefore unable to determine species-specific \(K_d\) values at \(I = 110\) mM for Na–KGa-1b.

**Effect of Exchangeable Cation.** To examine the influence of the type of exchangeable cation on SMZ association with montmorillonite clays, we conducted pH adsorption edge experiments using SWy-2 initially homoionic in Li\(^+\), Na\(^+\), K\(^+\), Mg\(^{2+}\), and Ca\(^{2+}\) (\(I = 10\) mM). Symbols represent means of triplicate measurements; error bars indicate one standard deviation. Trendlines represent best fits to eq 2.

![FIGURE 3. Adsorption edge for SMZ sorption to SWy-2 initially homoionic in Li\(^+\), Na\(^+\), K\(^+\), Mg\(^{2+}\), and Ca\(^{2+}\) (\(I = 10\) mM). Symbols represent means of triplicate measurements; error bars indicate one standard deviation. Trendlines represent best fits to eq 2.](image)

For the monovalent metal cations, \(K_{\text{SMZ-}\text{Na}}\), the trend in \(K_d\) values at pH 35 was not improved by inclusion of a term for SMZ adsorption to Ca–SWy-2 exceeded that to Na–SWy-2. The size of available adsorption domains on the siloxane surface was a significant determinant of SMZ\(^-\) sorption. Formation of π-complexes between basal plane oxygens and either the aniline or 4,6-dimethyl-pyrimidine rings of SMZ does not appear important because the tetrahedral \(-\text{SO}_2-\) group prevents close, conformational interaction of either aromatic moity with the siloxane surface. Possible mechanisms of SMZ\(^-\) interaction with the smectite surface include water and cation bridging. For the exchangeable cations examined in this study, aromatic amines such as aniline (pK\(_a\) = 4.6) interact with smectite surfaces primarily via water bridging (43). Because the anilinic amines of sulfonamide antimicrobials (pK\(_{a1}\) ≤ 2.3) are weaker bases than aniline, water-bridging rather than surface protonation represents a possible mechanism of interaction with montmorillonite between pH 5 and 7. Consistent with a water bridging mechanism via the anilic group, Ca\(^{2+}\) and Mg\(^{2+}\) saturation of the smectite enhanced SMZ\(^-\) adsorption over saturation with Na\(^+\), and adsorption to Ca–SWy-2 exceeded that to K–SWy-2 (43). Alternatively, SMZ\(^-\) could complex exchangeable cations through a pyrimidine N and/or the \(-\text{SO}_2-\) group. Pyrimidine forms inner and outer sphere complexes with exchangeable metal cations on montmorillonite surfaces (44). Bidentate coordination by a pyrimidine N and the \(-\text{SO}_2-\) group has been proposed for the solution complexation of divalent transition metal cations by SMZ and sulfadiamidine (45, 46). Further evidence from spectroscopic studies is needed to distinguish among these hypothesized mechanisms of interaction with smectite surfaces.

Cationic SMZ dominated adsorption to smectite surfaces at bulk solution pH values 1.5–2.5 units higher than expected based on pK\(_{a1}\) depending on the nature of the exchangeable cation. This was apparently due to increased occupation of exchange sites by protons as solution pH dropped below ~6 and enhanced dissociation of water solvating the exchange ion (35), leading to protonation of the aromatic amine at the smectite surface. Sulfamethazine adsorption to montmorillonites at low pH may have been further enhanced by protonation of the pyrimidine R group. The dissociation constant of the heterocyclic moiety appears to be <1.9 (47) and to our knowledge, has not been determined experimentally. Thus, adsorption of a SMZ dication may have contributed to the high \(K_d\) values observed at low pH. As noted above, the effects of ionic strength and the nature of the exchangeable cation were consistent with a contribution of a cation exchange mechanism to SMZ\(^-\) adsorption to smectites. Adsorption domain size also influenced the degree of SMZ\(^-\) sorption to smectites.

The data on SMZ adsorption to kaolinite do not allow us to confidently posit a mechanism of interaction. However, a number of observations can be made. Kaolinite particles present three types of surfaces to solution: a siloxane surface with a small amount of permanent negative charge, a gibbistic surface exposing aluminol groups, and edge sites displaying pH-dependent charge due to the protonation/deprotonation of silanol and aluminium groups. Only the neutral SMZ species appeared to interact with kaolinite. That SMZ\(^-\) did not contribute to adsorption at the pH values examined is consistent with the low permanent negative charge of KGa-1b, the dissociation constants of ionizable kaolinite surface functional groups, and the properties of water associated with kaolinite surfaces. Sulfamethazine adsorption to kaolinite was nearly invariant over a broad range of proton activities (pH 4.3–7), suggesting little, if any, interaction of SMZ\(^-\) with surfaces exhibiting variable charge over this pH range (i.e., edge sites). Surface-area-normalized \(K_d\) values for Na–KGa-1b exceeded those for the montmorillonites over the pH range where SMZ\(^-\) dominated solution speciation (p < 0.05), consistent with the notion that lower permanent
negative charge density on kaolinite siloxane surfaces provided larger domains for SMZ\textsuperscript{2}\textsuperscript{−} adsorption. We find it interesting that increased ionic strength resulted in a dramatic decrease in sulfamethazine sorption, a result that contrasts with the limited influence of ionic strength on the sorption of SMZ\textsuperscript{2}\textsuperscript{−} to montmorillonite siloxane surfaces. From our experimental data, we cannot exclude SMZ\textsuperscript{2}\textsuperscript{−} association with the gibbsitic surface of kaolinite. The manner in which increased NaCl concentration prevented interaction with kaolinite is not obvious. Clearly, further experimentation will be required to propose a compelling mechanism of sulfanamide interaction with kaolinite.

**Influence of Sulfanamide Structure on Adsorption.** To investigate the influence of the sulfanamide antimicrobial R group on adsorption to smectite surfaces, we conducted pH adsorption edge experiments with three sulfua drugs (viz. SMZ, SMX, and SPY) and fitted the experimental data to eq 2 to obtain species-specific $K_d$ values (Table 6). The sulfanilamide portion of these molecules is identical; the nature of the heterocyclic R group confers differences in physicochemical properties (Table 1). All three sulfonamides exhibited qualitatively similar trends in pH-dependent adsorption to Na--SWy-2 (Figure 4). The $pK_a$ values of SMX are much lower than those of SMZ and SPY, resulting in little sorption over most of the pH range examined. On the basis of fits of eq 2 to the experimental data, $K_d^{SA}$ >> $K_d^{SA}$ for all three sulfonamides (Table 6). The $K_d^{SA}$ value was largest for SMZ; those for SPY and SMX were 10 and 4-fold smaller. The $K_d$ values for SMZ\textsuperscript{−} and SPY\textsuperscript{−} adsorption to Na--SWy-2 were statistically indistinguishable ($p > 0.05$) due to the high variance in the SPY data; that of SMX\textsuperscript{−} was more than an order of magnitude smaller than those of SMZ\textsuperscript{−} and SPY\textsuperscript{−} ($p < 0.05$).

No clear relationship was apparent between modeled species-specific adsorption coefficients and compound hydrophobicity (as assessed by $K_{ow}$), but $K_d^{SA}$ values appeared to increase with molecular surface area (Table 1). Mechanisms of SPY and SMX interaction with the smectite surface are likely similar to those of SMZ. As was the case with SMZ, the $-\text{SO}_2-$ groups of these sulfonamides restrict interaction of the $\pi$-electron systems of aromatic moieties with the siloxane surface. Water and/or cation bridging represent likely mechanisms of interaction with montmorillonite. The trend in the magnitude of $K_d^{SA}$ paralleled that reported for stability constants of these compounds with divalent transition metal cations (45). The existence of a dicaticionic species is possible for both SMX and SPY. We were unable to find a study reporting a $pK_a$ value for the SPY pyridinium group, but work on structurally related methanesulfonamidopyridines (48) suggests that this moiety possesses a dissociation constant below that of the anilinium cation of sulfapyridine. As was the case for SMZ, adsorption of a SPY dication may have contributed to the large $K_d^{SA}$ value for this compound. For SMX, protonation of the isoxazole group likely occurs only at very low pH, below the dissociation constant for the anilinium group ($pK_{a1} = 1.8$). The relatively low $K_d^{SA}$ value may therefore reflect negligible contribution by the SMX dication at the proton activities examined.

Several previous investigators examined SMZ and SPY sorption to whole soils and soil clay fractions. Sulfamethazine sorption to homoionic SWy-2 and kaolinite exceeded that reported by Thiele-Bruhn et al. (13) to whole soils and soil clay fractions ($K_d = 2.4$ and 2.7 L kg\textsuperscript{−1}) and by Langhammer (49) to whole soils ($K_d = 0.89$ and 3.47 L kg\textsuperscript{−1}); the $K_d$ values for SMZ reported by these authors were similar to those we obtained for Na--SAz-1. Sulfapyridine sorption coefficients for whole soils and soil clay fractions (13, 50) were of the same order as those for Na--SWy-2.

**Adsorption Isotherms.** We examined SMZ adsorption to near homoionic SWy-2 as a function of sorbate concentration at pH 5.2 ± 0.1, a proton activity at which the neutral form comprised >99% of solution species and was the main contributor to adsorption (76--83%). Initial solute concentrations spanned 6 orders of magnitude (3.61 × 10\textsuperscript{−10} to 3.61 × 10\textsuperscript{−4} M). Isotherms for SMZ adsorption to SWy-2 near homoionic in Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, and Mg\textsuperscript{2+} (Figure 3S) were fit with the van Bemmelen--Freundlich equation:

$$
\log C_s = n \log C_w + \log K_f
$$

where $C_s$ (mol kg\textsuperscript{−1}) and $C_w$ (mol L\textsuperscript{−1}) are the sorbed and dissolved concentrations; $K_f$ is the Freundlich constant, a measure of sorption capacity at a specific aqueous concentration; and the Freundlich exponent $n$ provides a measure of the heterogeneity of adsorption site energies (51). The Freundlich coefficient is related to $K_d$ by the following equation:

$$
K_d = K_f C_w^{r_n-1}
$$

When $n = 1$, the isotherm is linear, sorption free energies at all sorbate concentrations are constant (51), and $K_d = K_f$. Table 7 summarizes Freundlich parameters for SMZ adsorption to initially homoionic SWy-2. As expected from pH sorption edge experiments, the type of exchangeable cation exerted little effect on SMZ adsorption. Adsorption isotherms

<table>
<thead>
<tr>
<th>sorbent</th>
<th>pH</th>
<th>$n$</th>
<th>$K_f$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na--SWy-2</td>
<td>5.05</td>
<td>1.01±0.00</td>
<td>13.7±1.0</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Mg--SWy-2</td>
<td>5.05</td>
<td>0.96±0.01</td>
<td>15.6±1.1</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>K--SWy-2</td>
<td>5.15</td>
<td>0.97±0.01</td>
<td>10.2±1.1</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Ca--SWy-2</td>
<td>5.10</td>
<td>1.01±0.01</td>
<td>10.7±1.0</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Na--KGA-1b</td>
<td>5.02</td>
<td>0.85±0.01</td>
<td>11.1±1.0</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* Values reported as mean ± standard error.

\[ TABLE 6. Effect of the Sulfonamide R Group on Adsorption to Na--SWy-2 \]

<table>
<thead>
<tr>
<th>sorbate</th>
<th>$K_d^{SA}$ (L kg\textsuperscript{−1})</th>
<th>$K_d^{SA}$ (L kg\textsuperscript{−1})</th>
<th>$\rho^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMZ</td>
<td>2620 ± 250</td>
<td>12.0 ± 0.7</td>
<td>0.96</td>
</tr>
<tr>
<td>SMX</td>
<td>160 ± 20</td>
<td>2.9 ± 0.1</td>
<td>0.99</td>
</tr>
<tr>
<td>SPY</td>
<td>3350 ± 1420</td>
<td>4.0 ± 0.3</td>
<td>0.95</td>
</tr>
</tbody>
</table>

* Values represent best fits of experimental data to eq 2 (mean ± standard error).

\[ TABLE 7. Freundlich Parameters for SMZ Adsorption to Near Homoionic Smectite and Kaolinite \]
for SWy-2 were linear, or nearly so, over the concentration range examined (i.e., $n = 0.96–1.01$), indicating that SMZ did not saturate available adsorption sites. Solubility limitations prevented investigation of higher SMZ concentrations.

The SMZ adsorption isotherm for Na–KGa-1b departed from linearity at high sorbate concentrations (Figure S4) with a Freundlich exponent of 0.85 (Table 7). The isotherm was well not fit by a Langmuir model, and the relatively low aqueous solubility of SMZ prevented extension of the isotherm to higher concentrations to examine approach to sorption capacity. The highest solution concentration examined resulted in <5% coverage of the kaolinite surface assuming monolayer coverage.

The linearity of the SWy-2 isotherms contrasts with the pronounced nonlinearity observed by some investigators examining sulfonamide sorption to whole soils and particle-size fractions. Thiele-Bruhn et al. (13) obtained markedly nonlinear isotherms for SPY sorption to clay fractions of loess Chernozem soils ($n = 0.81–0.84$) and attributed this to specific interaction with organic matter functional groups. These Freundlich coefficients are similar to those we obtained for SMZ adsorption to kaolinite. SPY sorption to whole soils exhibited a range of values for the Freundlich exponent (0.54–1.21), attributed by the authors to soil organic matter properties (13, 15, 50). In contrast to this, Boxall et al. (15) obtained Freundlich coefficients of 0.97 and 0.91 for the sorption of sulfachloropyridazine to clay loam and sandy loam. The soil mineralogy was not reported.

**Environmental Implications.** Accurate prediction of sulfonamide antimicrobial mobility and bioavailability in soils and subsurface environments requires a quantitative description of sorption as a function of aqueous phase composition. Our adsorption edge experiments and modeling of species-specific distribution coefficients highlight the importance of considering sulfonamide speciation in environmental fate modeling and enable estimation of adsorption to clay minerals over a range of pH and ionic strength conditions. Sulfonamide sorption to clay minerals is relatively insensitive to pH over the range of proton activities at which the neutral species dominates solution speciation. At higher and lower pH values, proton activity has a pronounced affect on sorption as the anionic and cationic species assume greater importance to overall sorption. Extrapolation of our results to real soils and sediments must be done with caution, as clays in such systems are often coated with organic matter and metal oxides. Our results demonstrate the importance of surface charge density for sulfonamide sorption to clay minerals. The surface charge densities of soil and sediment clays likely differ from those of the reference clays we employed. Sorption to soil and sediment clays is expected to vary from that observed for the reference clays in a manner that can be rationalized from this study.

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**Supporting Information Available**

Chemical purity and supplier information; sulfonamide properties and speciation; clay preparation and characterization; batch adsorption experiments; high-performance liquid chromatography method; liquid scintillation counting method; X-ray diffractometry method; smectite $d_{001}$ spacings after sulfonamide adsorption; SMZ adsorption to kaolinite; SMZ adsorption isotherms. This material is available free of charge via the Internet at http://pubs.acs.org.

**Literature Cited**


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