TETRACYCLINE SORPTION TO COAL AND SOIL HUMIC ACIDS: AN EXAMINATION OF HUMIC STRUCTURAL HETEROGENEITY

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Abstract—The sorption properties of tetracycline were compared between a coal humic acid (CHA) and a soil humic acid (SHA) under various solution chemistry conditions. Structural characteristics of the two humic acids were quantified in detail by elemental analysis, solid-state $^{13}$C nuclear magnetic resonance (NMR), acid-base titration, and Zeta potential measurement. The CHA consists primarily of poly(methylene)-rich aliphatics with more aromatic C–O and higher negative surface charge, while SHA is mainly composed of young material residues of lignin, carbohydrates and peptides, and oxidized charcoal. At pH 5 the sorption affinities of tetracycline to SHA and CHA were very close, but remarkably stronger than that to functionality-free model polymeric sorbents (polyethylene and polystyrene). Meanwhile, despite the much lower hydrophobicity, tetracycline displayed stronger sorption to the humic acids than nonpolar, nonionized 1,3,5-trichlorobenzene. It is thus concluded that specific complexation (H–bonding and cation exchange) with the humic functionality overwhelmed hydrophobic effect in sorption when tetracycline was dominated by the zwitterion. Furthermore, modifying solution chemistry conditions (pH, ionic strength of NaCl and CaCl$_2$, and the presence of Zn$^{2+}$) generally caused more prominent effects on tetracycline sorption to CHA than to SHA, which was attributed to the higher surface charge of CHA. Results of this research demonstrate the importance of the structural nature of humic acids in antibiotic sorption. Environ. Toxicol. Chem. 2010;29:1934–1942.

INTRODUCTION

Tetracycline antibiotics are produced in large quantity and heavily used in the farming industry [1]. Due to the relatively low metabolism and absorption by the animal body, most of the tetracyclines administered are excreted via feces and urine as unmodified parent compounds and consequently released into the environment [2–4]. Residues of veterinary pharmaceuticals, including tetracyclines discharged from agricultural runoff, are frequently detected in soil [5–7]. For example, tetracycline concentrations in a pig slurry drained clay soil were as high as 1,691 μg/kg [7]. The presence of antibiotic residues in the soil environment has raised significant concerns over a wide variety of adverse effects, including acute and chronic toxicity and microorganism antibiotic resistance [8,9].

Sorption to soil is a key process controlling the reactivity and mobility of organic contaminants in surface and subsurface environments. For nonpolar organic compounds such as polycyclic aromatic hydrocarbons (PAHs), sorption to soil is dominated by hydrophobic partition into soil organic matter (SOM), while inorganic soil components (clay minerals, iron/aluminum oxides, quartz, and feldspars) play only a minor role [10–12]. Alternatively, many pharmaceutical antibiotics such as tetracyclines are amphoteric molecules having multiple polar/ionized groups (hydroxyl, amino, and ketone), and hence are expected to interact strongly with inorganic soil components that are highly polar/charged. Several important specific mechanisms, including cation exchange and surface complexation via H–bonding and cation bridging, have been identified in previous studies on tetracycline sorption to soil minerals (smectite clays and aluminum oxides) [13–16].

Humic acids are operationally defined as alkali-soluble but acid-insoluble components in the SOM matrix and act as an important sorption phase for organic contaminants. Thus far, very limited studies have been performed to investigate tetracycline sorption to purified humic acids. Recently, Gu et al. [17] modeled tetracycline as a monooacid capable of interacting with discrete deprotonated sites in humic acids by ligand exchange, and provided reasonable prediction performance on tetracycline sorption. Notably, the ligand exchange mechanism of cationized species may be heavily regulated by solution chemistry conditions (pH, ionic strength, and cosolute of heavy metal ions). Changing pH might cause protonation-deprotonation transition of the functional groups in tetracycline molecules and humic acids, therefore influencing their mutual interactions and the sorption properties of tetracycline [17]. Similarly, the binding reactivity of both tetracycline molecules and humic ligands could be modified by the electronic screening effect as ionic strength changes. Furthermore, amendment of heavy metal ions such as Cu$^{2+}$ to clay minerals and organic matters was found to enhance tetracycline sorption due to ternary complex formation via cation bridging [18,19].

It is noteworthy that, depending on the source, diagenesis, and extraction procedures, humic acids are highly heterogeneous in structure [20–23]. For example, compared with aquatic humic acids terrestrial humic acids generally have higher molecular weight and larger content of aromatic carbon [20]. It is well recognized that the structural nature of humic acids exerts significant impact on sorption of nonpolar organic contaminants [22–25]. For example, sorption of PAHs and...
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chlorinated benzenes to humic substances was reported to be negatively related to the polarity of sorbent, simply indexed as the atomic ratio of (O+N)/C [11,22,26]. In a recent study by Sun et al. [27] it was shown that the structural properties of organic solutes and humic acids exhibit combined effects on sorption. Sorption of nonpolar and more hydrophobic solutes (phenanthrene, tetrachlorobenzene) was much greater to a mature coal humic acid with low polarity than to a young soil humic acid with high polarity, due to the predominance of a hydrophobic effect; however, disparities in sorption affinity between the two humic acids were much smaller for a polar and less hydrophobic solute (dichlorophenol) because polar interactions played an important role in sorption. However, thus far few studies have been carried out to investigate the impact of the structural heterogeneity of humic acids on sorption of pharmaceutical antibiotics.

The overall goal of the present study was to systematically examine the mechanisms and key factors controlling tetracycline sorption to humic acids. The sorption behavior of tetracycline under various solution chemistry conditions was compared between a coal humic acid (CHA) and a soil humic acid (SHA) using the batch sorption approach. Structural characteristics of the two humic acids were quantified in detail by elemental analysis, solid-state $^{13}$C nuclear magnetic resonance (NMR), acid-base titration, and Zeta potential measurement to correlate the sorption properties of tetracycline with the molecular nature of humic acids.

MATERIALS AND METHODS

Materials

A udic argosol was collected from the surface horizon (0–20 cm) from Shenyang, Liaoning Province in northeast China. The soil was air-dried, sieved (<2 mm), and stored at room temperature. Results of texture analysis, mineralization composition, organic carbon content (2.33%, dry wt based), and cation exchange capacity ([CEC], 19.7 cmol/kg) of the soil were given in our previous studies [12,28]. An SHA was extracted from the soil using a method similar to that in previous studies [27,29]. The soil was air-dried and passed through a 2-mm sieve, and was further treated with 0.2 M HCl to remove carbonates. After centrifugation, the obtained soil was mixed with 0.5 M NaOH and was continuously stirred under an atmosphere of N$_2$ at room temperature for 24 h. Then the soil suspension was centrifuged and the supernatant was collected and acidified with 6 M HCl (with the final pH adjusted to 1.5) to precipitate the humic acid, followed by washing with a mixture of 0.06 M HCl and 0.14 M HF to remove mineral residues. The humic acid solid was then dissolved with 0.1 M NaOH and neutralized with 1 M HCl. The resulting humic acid solution was dialyzed using membrane tubes (500 Da, Union Carbide) and freeze-dried. The obtained humic acid was stored in a sealed vial for later use. A CHA was purchased from Fluka with a further deashing treatment by HCl/HF using the method mentioned above. Polyethylene beads (medium density/332119, Aldrich) and polystyrene beads (331651, Aldrich) (ground before use) were included as additional model sorbents that are free of polar functionalities.

The two test sorbate compounds were tetracycline (hydrate, 99%, International Laboratory) and uncharged, nonpolar 1,3,5-trichlorobenzene (99%, Supelco). Their solute aqueous solubility ($S_w$), $n$-octanol-water partition coefficient ($K_{OW}$), and acid dissociation constants ($pK_{a,s}$) are listed in Table 1. The chemical structure of tetracycline is provided in Figure 1.

Characterization of HAs

Elemental analysis. Elemental analyses were performed using an elemental analyzer of Heraeus CHN-O-RAPID to determine the C, H, N contents of the two humic acids.

$^{13}$C NMR spectroscopy. The experiments were performed on a Bruker Avance 400 spectrometer with 4-mm sample rotors in a double-resonance probe head at 100 MHz using the following three techniques. First is the high-speed quantitative $^{13}$C direct polarization/magic angle spinning (DP/MAS) NMR. This technique provides quantitative structural information and was run at a spinning speed of 14 kHz. The 90° $^{13}$C pulse-length was 4 s. Recycle delays were tested by the cross-polarization/spin-lattice relaxation time–total sideband suppression (CP/T1-TOSS) technique to ensure that all carbon sites were fully relaxed. The details of this technique have been described elsewhere [30]. Second is the $^{13}$C cross-polarization/total sideband suppression (CP/TOSS) and $^{13}$C CP/TOSS with dipolar dephasing. Qualitative composition information was obtained with good sensitivity by $^{13}$C CP/TOSS NMR experiments at a spinning speed of 5 kHz and a CP time of 1 ms, with a 1H 90° pulse-length of 4 μs and a recycle delay of 1 s. Four-pulse total suppression of sidebands (TOSS) [31] was employed before detection, and two-pulse phase-modulated (TPPM) decoupling was applied for optimum resolution. The corresponding subspectrum with signals of nonprotonated carbons and mobile groups such as rotating CH$_3$ was obtained by $^{13}$C CP/TOSS combined with 40-μs dipolar dephasing. Third is the $^{13}$C chemical-shift-anisotropy (CSA) filter. To separate the signals of anomic carbons (O–C–O) from those of aromatic carbons (both of which may resonate between 120 and 90 ppm) the aromatic-carbon signals were selectively suppressed by a five-pulse $^{13}$C chemical-shift-anisotropy (CSA) filter with a time of 35 μs [32].

<table>
<thead>
<tr>
<th>Compound</th>
<th>$S_w$ (mmol/L)</th>
<th>$K_{OW}$ (L/L)</th>
<th>$pK_{a,s}$a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3,5-trichlorobenzene</td>
<td>0.0295b</td>
<td>15500b</td>
<td>NAc</td>
</tr>
<tr>
<td>tetracycline</td>
<td>3.8c</td>
<td>0.0646c</td>
<td>3.30(1), 7.68(2), 9.69(3)</td>
</tr>
</tbody>
</table>

a Tolls [5].
b Schwarzenbach et al. [45].
c Not applicable.

Table 1. Sorbate water solubility ($S_w$), $n$-octanol-water partition coefficient ($K_{OW}$), and sequential acid dissociation constants ($pK_{a,s}$)

Fig. 1. Chemical structure of tetracycline. The regions framed by dashed lines represent the structural moieties associated with the three acidic dissociation constants ($pK_{a,s}$).
Acid-base titration. Acid-base titration experiments were conducted to determine the carboxyl and phenolic hydroxyl groups of the humic acids. A solution of 0.05 M HCl or NaOH was added continuously at 100 µl (equivalent to 5 micromole) each time under an atmosphere of N₂ to a humic acid sample suspended in deionized water (20 mg humic acid in 50 ml water) in a thermostatic cell at 25°C. The suspension pH was equal to 4.04 for SHA and 4.80 for CHA prior to titration. The carboxyl content was estimated as the total consumption of alkali between the initial pH and pH 9.0 and acid between the initial pH and pH 3.0, and the phenol content was estimated as twice the alkali consumption between pH 8.0 and 10.0 [33,34].

Zeta potential. Zeta potential of the two humic acids prepared by aqueous suspension with 0.02 M NaCl (400 mg humic acid per liter solution) as a function of pH (adjusted with 0.1 M NaOH and HCl) was measured on duplicate samples using a Zeta potential analyzer (Brookhaven Instruments).

Batch sorption

The aqueous solution of 0.02 M NaCl containing tetracycline at the desired concentration was preadjusted for pH with 0.1 M NaOH and HCl and purged by N₂ to remove dissolved oxygen to prevent possible oxygen-mediated degradation of tetracycline [35]. A 22-ml amber U.S. Environmental Protection Agency vial equipped with a polytetrafluoroethylene-lined screw cap received a weighed amount of sorbent (15–20 mg of humic acids, 50 mg of polyethylene or polystyrene) and a full volume of aqueous tetracycline solution and aqueous stock solution of Zn²⁺, if needed, or aqueous background solution (0.02 M NaCl) followed by a methanol stock solution of 1,3,5-trichlorobenzene. The initial concentration of Zn²⁺ was approximately equal to 16.5 mg/L. If methanol was used, its volume ratio to aqueous solution was kept below 0.1% to minimize cosolvent effects. The samples were shielded from light using aluminum foil to avoid possible photodegradation, and were mixed end-over-end at room temperature for 3 d for all sorbate/sorbent combinations. The time was used to reach apparent sorption equilibrium (no further uptake) based on predetermined adsorption kinetics. The sample pH was adjusted with 0.1 M NaOH and 0.1 M HCl and measured to be 5.0 ± 0.1 at the end of batch sorption experiments. Separate sets of single-point experiments were conducted to study the effect of pH ranging from 2.2 to 6.7, and the effect of ionic strength (NaCl or CaCl₂) ranging from 0.01 to 0.1 M on tetracycline sorption. Triplicate samples were performed for the ionic strength experiments and duplicate samples were performed for all other experiments.

After centrifugation, solute 1,3,5-trichlorobenzene was extracted from an aliquot of the aqueous phase with hexanes and analyzed by gas chromatography with electron-capture detection using a 60 m × 0.25 mm DB-1 capillary column (J&W Scientific). Solute tetracycline was analyzed directly by high-performance liquid chromatography with an ultraviolet detector using a 4.6 × 150 mm HC-C18 column (Agilent, USA). Isocratic elution was performed under the following conditions:

0.01 M oxalic acid–acetonicitrile–methanol (80:16:4, v:v:v) with a wavelength of 360 nm. To account for solute loss from processes other than sorbent sorption (sorption to glassware and septum and volatilization), calibration curves were obtained separately from controls receiving the same treatment as the sorption samples but no sorbent. Calibration curves included at least seven standards over the test concentration range. When electron-capture detection was used for analysis, calibration curves were fit to a power law expression to account for the detector response nonlinearity. Based on the obtained calibration curves, the sorbed mass of solute was calculated by subtracting mass in the aqueous phase from mass spiked.

RESULTS AND DISCUSSION

Characterization of HAs

Elemental composition. Elemental compositions of C, H, N of the two humic acids are given in Table 2. The SHA has a larger nitrogen content than CHA but other elemental contributions were within 8.0% difference.

$^{13}$C NMR spectroscopy. The CHA was characterized in detail by Sun et al. [27]. Therefore, only its quantitative DP/MAS was included in the present study to facilitate comparison with that of SHA (Fig. 2a). The DP/MAS spectrum of SHA is displayed in Figure 2b. These two DP spectra provide quantitative structural information. The chemical shift ranges of the different functional groups are assigned in accordance with previous studies [30] and the respective percentages are listed in Table 3. The proportions of total spectral area differ noticeably between CHA and SHA. Their structural differences were summarized after examining the detailed structural information of SHA based on spectral-editing techniques (see below).

Figure 2c–e shows $^{13}$C CP/MAS NMR spectra for SHA acquired with spectral-editing techniques to select subspectra of specific types of chemical groups. Figure 2c is $^{13}$C CP/TOSS spectrum, which shows qualitative structural information and is used primarily as a reference spectrum for two other spectral-editing spectra. Compared with Figure 2b, the region from approximately 100 to 200 ppm assigned to sp²-hybridized carbons of Figure 2c is significantly reduced, whereas that from approximately 0 to 100 ppm is markedly enhanced, primarily due to significant signals of sp²-hybridized carbons lost in sidebands due to their high chemical shift anisotropies, and low cross-polarization (CP) efficiencies of sp²-hybridized carbons is due to their dominantly nonprotonated carbons and high CP efficiencies of sp³-hybridized carbons is due to their significant protonated carbons. Therefore, CP is not a quantitative technique and it is necessary to acquire DP spectra to obtain quantitative spectra. The corresponding $^{13}$C CP/TOSS spectrum after 40 µs of dipolar dephasing (Fig. 2d) shows solely signals of nonprotonated carbons and mobile groups, including rotating CH₃ groups, which have a reduced C–H dipolar coupling due to their fast motion. It shows merely small signals from highly mobile CH₃ (10–24 ppm) and –(CH₂)n– (≈30 ppm) components, and OCH₃ groups (≈56 ppm). Most of the carbohydrates are protonated because signals between 60

<table>
<thead>
<tr>
<th>Humic acid</th>
<th>Elemental composition (wt%)</th>
<th>Carboxyl (meq/g)</th>
<th>Phenol (meq/g)</th>
<th>Total acidity (meq/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHA</td>
<td>Oxygen 58.20</td>
<td>Hydrogen 4.26</td>
<td>Nitrogen 2.88</td>
<td>4.82</td>
</tr>
<tr>
<td>SHA</td>
<td>Oxygen 54.65</td>
<td>Hydrogen 4.60</td>
<td>Nitrogen 5.77</td>
<td>5.94</td>
</tr>
</tbody>
</table>
and 95 ppm barely show in the dipolar-dephased spectrum (Fig. 2d). In addition, nonprotonated aromatic carbons are detected at 130 ppm, aromatic C–O between 144 and 162 ppm, COO/N–C––O groups at approximately 173 ppm, and ketones between 190 and 200 ppm. Note that the signals between 190 and 200 ppm in the CP/TOSS (Fig. 2c) and also DP/MAS (Fig. 2b) belong primarily to ketones, not aldehydes, because they are mostly retained in the dipolar-dephased spectrum (Fig. 2d). The presence of signals at approximately 56 ppm and between 144 to 162 ppm indicates that SHA contains lignin residues. The dominant signals at approximately 130 ppm could be from residues of charcoal. This information combined with significant signals of COO suggests the occurrence of oxidized charcoal in this sample, similar to a grassland SHA investigated before [36]. The significant signal loss for the dipolar-dephased spectrum from approximately 50 to 64 ppm is indicative of the presence of NCH. The NCH combined with the presence of N–C––O at approximately 173 ppm indicates the presence of peptide residues. The CP/TOSS spectrum after a 13C CSA filter of 35 μs, which exhibits only sp3-carbon signals, is displayed in Figure 2e. In particular, this technique separates overlapping anomers (O–C–O) from aromatics between 90 to 120 ppm. A clear O–C–O band is displayed at approximately 100 ppm in this region (Fig. 2e), clearly demonstrating that the sample contains carbohydrate residues.

In summary, SHA contains at least four major components: lignin residues, carbohydrate residues, peptide residues, and oxidized charcoal residues. Based on our previous study [27], CHA consists primarily of nonpolar poly(methylene)-rich aliphatics which probably form a domain, high aromatics whose rings could also be fused, such as those in oxidized charcoal, lignin residues, ketones, and aldehydes. Unlike SHA, CHA does not contain obvious sugars. The quantitative data show that CHA contains more nonpolar alkyl, aromatic C–O, and ketones/aldehydes, but less O-alkyl, aromatics, and COO/N–C––O groups.

Acid-base titration. The carboxyl and phenol contents determined by acid-base titration are also presented in Table 2. Compared with CHA, SHA has a slightly higher carboxyl content, but slightly lower phenol content, consistent with

Table 3. Percentages of total spectral area assigned to different functional groups obtained by quantitative 13C direct polarization/magic angle spinning (DP/ MAS) nuclear magnetic resonance (NMR) for coal humic acid (CHA) and soil humic acid (SHA)

<table>
<thead>
<tr>
<th>Chemical shift (ppm)</th>
<th>220–188</th>
<th>188–162</th>
<th>162–144</th>
<th>144–93</th>
<th>110–64</th>
<th>64–50</th>
<th>50–0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assignment</td>
<td>CHA</td>
<td>SHA</td>
<td>CHA</td>
<td>SHA</td>
<td>CHA</td>
<td>SHA</td>
<td>CHA</td>
</tr>
<tr>
<td>Ketones, aldehydes</td>
<td>5.5</td>
<td>2.9</td>
<td>14.3</td>
<td>18.3</td>
<td>12.3</td>
<td>9.4</td>
<td>35.4</td>
</tr>
<tr>
<td>COO/N–C––O</td>
<td>13.4</td>
<td>12.3</td>
<td>13.4</td>
<td>9.4</td>
<td>11.8</td>
<td>3.6</td>
<td>24.4</td>
</tr>
<tr>
<td>Aromatic C–O</td>
<td>35.4</td>
<td>43.7</td>
<td>11.8</td>
<td>3.6</td>
<td>3.2</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td>Aromatics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-alkyl</td>
<td>4.9</td>
<td>4.9</td>
<td>4.9</td>
<td>4.9</td>
<td>4.9</td>
<td>4.9</td>
<td>4.9</td>
</tr>
<tr>
<td>N-alkyl, methoxyl</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Nonpolar alkyl</td>
<td>24.4</td>
<td>24.4</td>
<td>24.4</td>
<td>24.4</td>
<td>24.4</td>
<td>24.4</td>
<td>24.4</td>
</tr>
</tbody>
</table>

Sidebands are corrected. Anomeric carbons are separated from aromatic carbons based on the 13C chemical shift anisotropy (CSA)-filtered spectra. N=nitrogen; O=oxygen; C=carbon. The quantitative data of CHA from Fluka have been published in a study by Sun et al. [27] for a different objective.
the $^{13}$C NMR data. Nonetheless, the two humic acids have very close total acid content (carboxyl plus phenol).

**Zeta potential.** Figure 3 displays Zeta potential of the two humic acids as a function of pH. The two humic acids are always negatively charged and show similar trends of pH dependency within the test pH range of 2.2 to 6.7. The Zeta potential decreases with increase of pH up to approximately 5.7, and then remains nearly unchanged when the pH is further increased. Moreover, CHA is constantly more negatively charged than SHA by approximately 10 mV in terms of Zeta potential within the examined pH range, indicating that the functional groups (carboxyl, phenol) of CHA are more dissociated. This seems contradictory to the observations that, compared with CHA, SHA has higher carboxyl content and similar total acid content, as reflected in the $^{13}$C NMR and acid-base titration experiments. A plausible explanation for the seeming inconsistency is that the acid functional groups in CHA are probably more clustered/conjugated, and thus exhibit lower $pK_a$ values and easier deprotonation (similar to salicylate and phthalate). This hypothesis is supported by the fact that CHA is more mature and richer in cross-linked structures than SHA. It should also be pointed out that for soft polyelectrolyte substances such as humic acids the Zeta potential measured on the basis of electrophoretic mobilities may not reflect the surface charge appropriately [37].

**Batch sorption.**

**Sorption affinity.** Sorption isotherms plotted as solid-phase concentration ($q$) against aqueous-phase concentration ($C_0$) at sorption equilibrium for different sorbate/sorbent combinations are presented in Figure 4. The sorption data were fit to the Freundlich model ($q = K_f C_0^n$) by nonlinear regression weighed by $1/q$, where $K_f$ (mmol$^{1-n}$/L/kg) is the Freundlich affinity coefficient and $n$ (unitless) is the Freundlich linearity index. The Freundlich model parameters are summarized in Table 4, along with the upper and lower boundary values of the distribution coefficient ($K_d$) within the examined concentration ranges. In general, the Freundlich model provides good fits for the sorption data.

Several interesting results can be generalized based on comparison of the sorption data between different sorbate/sorbent combinations. First, despite the much lower hydrophobicity of tetracycline (as justified by the $S_W$ and $K_{OW}$ values; see Table 1), sorption of tetracycline is even stronger than 1,3,5-trichlorobenzene to the two humic acids, wherein the differences in $K_d$ are up to one order of magnitude (see Table 4). Second, sorption of tetracycline is much stronger to the two humic acids ($K_d > 1,300$ L/kg) than to the two model polymeric sorbents, polyethylene and polystyrene ($K_d < 300$ L/kg), while 1,3,5-trichlorobenzene exhibits reversed trends. These results are understandable considering the structural properties of both sorbates and sorbents. The tetracycline molecule has multiple polar functional groups, including hydroxyl, ketone, and amino, which may interact strongly with the respective functional groups in humic acids via H–bonding. Moreover, at the test pH of approximately 5.0 tetracycline is dominated by the zwitterion, and meanwhile a significant fraction of the carboxyl groups in humic acids are dissociated and negatively charged (see the Zeta potential results presented in Fig. 3), therefore leading to strong mutual interactions via electrostatic forces (cation exchange). In contrast, the 1,3,5-trichlorobenzene molecule is uncharged and contains no polar group, and hence cannot invoke the above-mentioned specific interactions with humic acids. Similarly, the same argument can be used to explain the very low sorption affinity of tetracycline to polyethylene and polystyrene that are nonpolar and free of polar functionality. However, the high hydrophobicity of 1,3,5-trichlorobenzene results in strong hydrophobic partition into polyethylene and polystyrene, with the $K_d$ as high as 4,000 L/kg (see Table 4). Thus, it can be concluded that sorption of tetracycline to humic acids is mainly driven by specific sorptive interactions (H–bonding, cation exchange), while the hydrophobic effect is only a minor cause. Similar specific mechanisms have also been proposed previously for tetracycline sorption to humic acids based on batch sorption experiments and model studies [17,38], as well as Fourier-transform infrared spectroscopic evidence [13].

In the present study the specific mechanisms of tetracycline sorption to humic acids can be further verified by the higher degree of sorption nonlinearity (reflected by more departure of the Freundlich $n$ value from 1, see Table 4), as compared with other sorbate/sorbent combinations. For example, the Freundlich $n$ value is 0.73 for tetracycline with SHA, 0.85 for 1,3,5-
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Table 4. Freundlich model parameters, $K_F$ and $n \pm$ standard deviation, and distribution coefficients ($K_d$) for sorption of tetracycline (TC) and/or 1,3,5-trichlorobenzen (TCB) to different sorbents.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sorbent$/\text{Electrolyte}^{b}$</th>
<th>$K_F \text{ mmol}^{1-n} \text{ L/kg}$</th>
<th>$n$</th>
<th>$R^2$</th>
<th>$K_d \text{ L/kg}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>SHA/Na$^+$</td>
<td>380 ± 70</td>
<td>0.73 ± 0.04</td>
<td>0.974</td>
<td>1300–2700</td>
</tr>
<tr>
<td>TC</td>
<td>SHA/Zn$^{2+}$</td>
<td>900 ± 200</td>
<td>0.83 ± 0.05</td>
<td>0.964</td>
<td>1600–3100</td>
</tr>
<tr>
<td>TC</td>
<td>CHA/Na$^+$</td>
<td>700 ± 100</td>
<td>0.76 ± 0.04</td>
<td>0.973</td>
<td>1700–3700</td>
</tr>
<tr>
<td>TC</td>
<td>CHA/Zn$^{2+}$</td>
<td>6000 ± 1000</td>
<td>0.98 ± 0.04</td>
<td>0.981</td>
<td>5500–9100</td>
</tr>
<tr>
<td>TC</td>
<td>PE/Na$^+$</td>
<td>400 ± 100</td>
<td>1.15 ± 0.07</td>
<td>0.963</td>
<td>170–290</td>
</tr>
<tr>
<td>TC</td>
<td>PE/Zn$^{2+}$</td>
<td>220 ± 60</td>
<td>0.98 ± 0.06</td>
<td>0.959</td>
<td>190–320</td>
</tr>
<tr>
<td>TC</td>
<td>PS/Na$^+$</td>
<td>80 ± 10</td>
<td>1.02 ± 0.04</td>
<td>0.986</td>
<td>60–100</td>
</tr>
<tr>
<td>TC</td>
<td>PS/Zn$^{2+}$</td>
<td>160 ± 40</td>
<td>1.06 ± 0.06</td>
<td>0.965</td>
<td>70–150</td>
</tr>
<tr>
<td>TCB</td>
<td>SHA/Na$^+$</td>
<td>60 ± 10</td>
<td>0.85 ± 0.04</td>
<td>0.979</td>
<td>100–210</td>
</tr>
<tr>
<td>TCB</td>
<td>CHA/Na$^+$</td>
<td>880 ± 90</td>
<td>0.91 ± 0.02</td>
<td>0.997</td>
<td>1400–2400</td>
</tr>
<tr>
<td>TCB</td>
<td>PE/Na$^+$</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>4000 ± 1000</td>
</tr>
<tr>
<td>TCB</td>
<td>PS/Na$^+$</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>540 ± 5</td>
</tr>
</tbody>
</table>

$^a$ SHA, soil humic acid; CHA, coal humic acid; PE, polystyrene; PS, polystyrene.

$^b$ Na$^+$, 0.02 M NaCl only; Zn$^{2+}$, 0.02 M NaCl plus Zn$^{2+}$ initially spiked at 16.5 mg/L. The equilibrium pH was approximately 5.0.

$^c$ Reported as upper and lower boundary values within the examined concentration ranges except where noted. The initial concentrations of tetracycline were the same for all sorbate/sorbent combinations, ranging from 1.80 × 10$^{-3}$ mmol/L to 4.62 × 10$^{-3}$ mmol/L.

$^d$ Not determined.

$^e$ Measured from single-point batch experiments with five or six replicate samples (TCB spiked at 6.01 × 10$^{-3}$ mmol/L).

A large disparity in sorption of 1,3,5-trichlorobenzene is observed between SHA and CHA; however, the difference is much smaller for tetracycline (Fig. 4). Structural characterization of the two humic acids by solid-state $^{13}$C NMR demonstrates that CHA consists primarily of nonpolar poly(methylene)-rich aliphatics which probably form a hydrophobic domain, while SHA consists primarily of young materials of residues of lignin, peptide, and sugars which have higher polarities. Accordingly, sorption of nonpolar 1,3,5-trichlorobenzene is stronger to CHA than to SHA, due to the predominance of hydrophobic effect. In contrast, tetracycline sorption to humic acids is controlled by specific interactions (H–bonding, cation exchange), and therefore the disparity in sorption between the two humic acids becomes much smaller. Consistent results have been shown in our previous study [27] when comparing sorption of nonpolar, highly hydrophobic solutes (phenanthrene, tetrachlorobenzene) and a highly polar, less hydrophobic solute (dichlorophenol) between CHA and another humic acid from a surface soil of Jiangsu Province in east China.

The sorption affinity of tetracycline correlates very well with the total acid content, but poorly with the surface charge (Zeta potential), polarity, and aromaticity of the two humic acids. Therefore, it seems that H–bonding interactions with the carboxyl and phenol groups in humic acids play a more important role than cation exchange, polar interactions with other functional groups (carbonyl and amide) and π–π electron donor-acceptor (EDA) interactions. At a pH of approximately 5.0, tetracycline is zwiterionized and does not possess any net charge, leading to a relatively weak cation-exchange interaction. However, it is noteworthy that cation exchange is still likely in effect between the cationized moiety of tetracycline and dissociated carboxyl groups. Due to the strong electron-withdrawing ability of the ketone groups, the substituted unsaturated moieties in tetracycline are electron deficient, and hence may act as π-electron-acceptors. Strong π–π EDA interactions have been verified between the tetracycline molecule and the polarized polycyclic aromatic rings (graphene) (π-electron-donor) on the surface of carbonaceous adsorbents (carbon nanotubes, graphite) [39]. A similar mechanism has also been identified between the oxytetracycline molecule and aromatic rings of humic acids coated on montmorillonites [13]. However, the content of char-like fused aromatic structures in the alkali-extracted, purified humic acids is expected to be very low. Moreover, the fused aromatic structures might also be blocked with other humic structural components (likely bearing aromatic moieties) from access to tetracycline molecules. In fact, adsorption of aromatic solutes on nonporous graphite was found to be remarkably suppressed by the presence of dissolved humic acids due to competitive adsorption for sites on the graphene surface [40,41].

Effect of Zn$^{2+}$ on sorption. Sorption isotherms of tetracycline to SHA, CHA, and polystyrene with and without the presence of Zn$^{2+}$ are compared in Figure 5. In the present study, Zn$^{2+}$ serves as a representative heavy metal ion for its environmental relevance to be micronutrients at low concentrations but toxicants at high concentrations. Prominent Zn$^{2+}$-enhanced sorption of tetracycline (up to 2.5 times) is shown for CHA, but the effect of Zn$^{2+}$ is negligible for SHA and polystyrene. A mechanism of cation-bridging has been proposed earlier to account for the sorption enhancement of tetracycline antibiotics to humic substances and mineral surfaces amended with strongly complexing metals (Cu$^{2+}$, Al$^{3+}$, Fe$^{3+}$) [18,19]. Likewise, this mechanism helps explain the Zn$^{2+}$-enhanced tetracycline sorption to CHA, in that ternary complexes are formed between Zn$^{2+}$ and tetracycline- and humic ligand groups. The higher sorption enhancement observed for CHA than for SHA can be attributed to the stronger metal-binding ability of CHA due to its higher surface charge (see Zeta potential results presented in Fig. 3). Consistently, the measured $K_d$ of Zn$^{2+}$ is higher to CHA (4,700 ± 700 L/kg) (based on single-point experiments with six replicates) than to SHA (2,650 ± 60 L/kg). Furthermore, as discussed above, compared with SHA, CHA may have more clustered/conjugated ligand groups (salicylate- and phthalate-like) with lower pK$_a$ values and easier deprotonation, which facilitate bidental coordination of heavy metal ions. In accordance with the cation-bridging mechanism, no sorption enhancement of tetracycline by Zn$^{2+}$ is observed for polystyrene because it contains no metal-complexing group.
Effect of pH on sorption. Figure 6 displays the effect of pH on tetracycline sorption to SHA, CHA, and polyethylene. Within the examined pH range of 2.2 to 6.7, SHA and CHA exhibit similar trends of pH-dependent sorption. The highest sorption occurs at a pH of approximately 3.6, and the sorption over the lower pH range (2.2–3.6) is consistently higher than the sorption over the higher pH range (3.6–6.7). These observations can be well explained by the combined effects of pH-mediated speciation of tetracycline and humic acid functional groups [13,17]. At pH 3.6, a significant fraction (33%) of tetracycline is cationized, and the rest is zwitterionized, leading to prominent interaction with the negatively charged functional groups (mainly carboxyl) of humic acids via cation exchange. Cation exchange interactions with humic substances are facilitated at optimal pH values when the cationized sorbate species and the dissociated humic carboxyl groups both reach the maximum amounts [42]. Additionally, it is evident that tetracycline sorption to CHA is always higher than that to SHA, and the disparity becomes even larger at the lower pH range (2.2–3.6). These results are also consistent with the cation exchange mechanism in consideration of the constantly higher surface charge (Zeta potential) of CHA than SHA within the examined pH range (Fig. 3). Moreover, the pH-dependent trend suggests that at low pH (<4.5 in the present study) cation exchange plays a more important role than other sorption mechanisms, including H–bonding in tetracycline sorption to humic acids. Unlike humic acids, polyethylene contains no proton-complexing group, and is thus essentially unaffected by pH.

Effect of ionic strength on sorption. The effect of ionic strength of NaCl or CaCl₂ on tetracycline sorption to CHA and SHA is presented in Figure 7. Several general trends can be observed. First, with the exception of Na⁺/CHA, tetracycline sorption to the two humic acids decreases with increasing ionic strength for both Na⁺ and Ca²⁺. Second, the effect of ionic strength on tetracycline sorption is much more pronounced for Ca²⁺ than for Na⁺–K⁺ changes several times for Ca²⁺, but less than 50% for Na⁺ over the examined range of ionic strength. Third, tetracycline sorption is stronger for Ca²⁺ than for Na⁺ at the ionic strength of 0.01 M, but reversed trends are observed for higher ionic strengths. To rationalize these observations, two mechanisms, cation-bridging and competitive sorption by the metal ions, both have to be considered. Tetracycline sorption to humic substances can be facilitated by multivalent cations such as Ca²⁺ through formation of the ternary complexes between the metal ions and tetracycline and humic functional groups [18]. Alternatively, due to the low

Fig. 6. Effect of pH on distribution coefficient (log \( K_d \)) for single-point sorption of tetracycline in 0.02 M NaCl to different sorbents: soil humic acid (SHA: \( \square \)), coal humic acid (CHA: \( \bigcirc \)), and polyethylene (PE: \( \triangle \)). Tetracycline was spiked at 1.68 \( \times 10^{-2} \) mmol/L for SHA and CHA, and 1.73 \( \times 10^{-2} \) mmol/L for PE. Error bars, in most cases smaller than the symbols, represent variability from duplicate samples. Lines are for visual clarity only.
complexing ability Na\(^+\) is incapable of invoking significant bridging interactions with humic acids. Thus, tetracycline sorption is more favored by Ca\(^{2+}\) relative to Na\(^+\) at low ionic strength (0.01 M). On the other hand, Ca\(^{2+}\) at higher concentrations may strongly compete for cationic exchange sites (mainly dissociated carboxyl groups) in humic acids, and hence cause a suppressive effect on tetracycline sorption. Note that the mechanism is also applicable to Na\(^+\), but as expected with much less efficiency due to the low complexing ability of Na\(^+\). Consistently, previous studies \cite{43,44} demonstrated that sorption of antibiotics (dodecylpyridinium, sulfachloropyridazine, tylosin, and oxytetracycline) to minerals and to soil decreased with an increasing concentration of Ca\(^{2+}\) or Na\(^+\), and attributed the observations to the competitive sorption of metal ions with the cationized antibiotics species for negatively charged sites. However, in contrast to SHA, tetracycline sorption to CHA is found to increase with increasing concentration of Na\(^+\), which is inconsistent with the proposed mechanism. Compared with SHA, CHA consists of highly cross-linked structural components with higher negative surface charge; therefore, CHA is more susceptible to change in conformation in response to an electrolyte alteration, leading to enhanced tetracycline sorption.

**CONCLUSIONS**

Due to specific sorptive interactions, both SHA and CHA exhibit very strong sorption affinity of tetracycline (\(K_d\) on the order of \(10^3\) L/kg), while the hydrophobic effect plays only a minor role in sorption. At pH approximately 5.0, the sorption affinity of tetracycline correlates very well with the total acid content (carboxyl plus phenol groups), but poorly with the surface charge (Zeta potential), polarity, and aromaticity of the two humic acids, suggesting the importance of H–bonding interactions between the zwitterionized tetracycline and the functional groups in humic acids. However, tetracycline sorption peaks at pH approximately 3.6 for the humic acids, wherein the cationized tetracycline species and dissociated carboxyl groups of humic acids both reach the maximum amounts to favor the cation exchange mechanism. Amendment of Zn\(^{2+}\) leads to prominent sorption enhancement of tetracycline to CHA, but causes little effect on sorption to SHA, attributed to formation of the ternary complexes between Zn\(^{2+}\), tetracycline, and the highly negatively charged CHA. Additionally, tetracycline sorption to the two humic acids is generally decreased by increasing the ionic strength of NaCl or CaCl\(_2\); resulting from competitive sorption of the metal ion for cationic exchange sites. Compared with SHA, the higher surface charge of CHA also causes more noticeable trends of proton– and Ca\(^{2+}\)-mediated sorption of tetracycline.

The findings in the present study highlight the importance of the structural nature of humic acids in tetracycline sorption. Compared with terrestrial humic acids, aquatic humic acids generally contain significantly fewer aromatics and more aliphatics and thus are characteristically distinct from the humic samples used in the present study. The sorption behavior of aquatic humic acids would be expected to be different and should also be examined in the future. The presence of multiple polar and ionizable groups in tetracyclines and other pharmaceuticals makes it difficult to sort out the nature of their sorptive interactions with humic substances. The simplified, single-parameter (\(K_{OW}\))-based linear partition model proposed for predicting the sorption behavior of low-polar, nonionic organic solutes would be problematic for pharmaceuticals. It should also be pointed out that information on pharmaceuticals on the sorbed complex at the molecular level is lacking but critically needed to complement that at the macroscopic level for a better understanding of the key factors governing the sorption mechanism.

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