Adsorptive removal of sulfonamide antibiotics in livestock urine using the high-silica zeolite HSZ-385

S. Fukahori, T. Fujiwara, N. Funamizu, K. Matsukawa and R. Ito

ABSTRACT

The adsorptive removal of seven sulfonamide antibiotics using the high-silica zeolite HSZ-385 from distilled water, synthetic urine and real porcine urine was investigated. The pH greatly affected the adsorption efficiency, and the amounts of all sulfonamide antibiotics adsorbed on HSZ-385 decreased at alkaline conditions compared with that at neutral conditions. During storage, the pH and ammonium-ion concentration increased with urea hydrolysis for porcine urine. We clarified that the adsorption efficiency of sulfonamides in synthetic urine was equivalent to that in distilled water, suggesting that adsorption behavior was not affected by coexistent ions. HSZ-385 could adsorb sulfonamide antibiotics in real porcine urine even though the non-purgeable organic carbon concentration of porcine urine was 4–7 g/L and was two orders of magnitude higher than those of sulfonamides (10 mg/L each). Moreover, the adsorption of sulfonamides reached equilibrium within 15 min, suggesting that HSZ-385 is a promising adsorbent for removing sulfonamides from porcine urine.

Key words | adsorption, high-silica zeolite, sulfonamide antibiotics, urine

INTRODUCTION

Sulfonamide antibiotics are very popular active antimicrobial agents used in the production of animal-based foods due to their relatively low cost; this is despite the introduction of new molecules such as penicillin or its derivates and other antibiotics (Hirsch et al. 1999). Recently, water pollution caused by pharmaceuticals has become a serious problem and attracted much concern (Ternes 1998; Hirsch et al. 1999; Kümmerer 2009). In agricultural research, sulfonamides are used as in-feed antibiotics and are excreted in urine and feces along with their metabolites (e.g. glucoronide and N-4-acetylated sulfonamides) (Garcia-Galán et al. 2008, 2010). Urine and feces from animals reared under intensive conditions are often spread as manure on pastures, and antibiotics could enter the environment through infiltration. Some sulfonamide antibiotics are not removed completely by microorganisms due to their high resistance to biodegradation (Ternes 1998). The eco-toxicology of pharmaceuticals at μg/L levels has been reported (Fent et al. 2006; Radjenović et al. 2009). Suitable treatment for the discharged pharmaceuticals is required for the sustainable use of water.

Adsorptive removal of pharmaceuticals is a promising technique. Adsorption of sulfonamides and other pharmaceuticals has been reported (Braschi et al. 2010). Zeolites are commonly used in wastewater and sewage treatment. Zeolites characterized by a low Si/Al ratio are hydrophilic. The hydrophilic zeolites such as A-type and F-type zeolites are used to remove cations by ionic exchange; however, they are not effective for the removal of organic contaminants in water. Our research team has also investigated the removal of sulfonamide antibiotics using the high-silica zeolite HSZ-385 (Fukahori et al. 2011). It was clarified that pH greatly affected the adsorption of sulfonamides because HSZ-385 can adsorb sulfonamides through hydrophobic interactions, and that the hydrophobicity of sulfonamides is dependent upon pH. The high-silica zeolite was a promising adsorbent for adsorptive removal of pharmaceuticals.
However, the effect of coexistent materials such as inorganic ions and organic matter on adsorption behavior was not investigated even though they are excreted mainly in urine along with their metabolites.

In the present study, adsorptive removal of sulfonamide antibiotics in distilled water and synthetic urine using a high-silica zeolite was achieved to evaluate the effect of ions. In addition, the composition of real porcine urine, its time-course during storage, and the adsorption behavior of sulfonamides in real porcine urine were investigated.

**METHODS**

**Materials**

High-silica Y-type zeolite (HSZ-385; surface area, 600 m²/g; mean particle size, 4 μm; SiO₂/Al₂O₃ = 100) was kindly provided by Tosoh Ltd (Tokyo, Japan). Seven sulfonamide antibiotics were selected as target compounds; sulfathiazole (STZ), sulfamethizole (SMthi), sulfamerazine (SMzi), sulfamethoxazole (SMzl), sulfadimethoxine (SMM), sulfadimidine (SDD). The details of quantitative analyses are shown in the Appendix section (see http://www.iwaponline.com/wst/067/513.pdf). The composition of synthetic urine was obtained from a previous related study (Wilsenach et al., 2007). Real porcine urine was collected from pigs without contamination of feces and stored at 4 °C after filtration. The concentration of ions in synthetic urine and porcine urine is given in Table 1.

**Adsorption experiment**

The adsorptive removal of sulfonamide antibiotics was undertaken. Briefly, a sulfonamide mixture solution (10 mg/L each; 5 mL) was prepared using distilled water, synthetic urine and porcine urine. Porcine urine was filtered with a membrane filter (Dismic; pore size, 0.20 μm; Advantec, Ltd., Tokyo, Japan) before use. The pH was adjusted to the desired value throughout the experiment using sulfuric acid and sodium hydroxide with measurement by a portable pH meter (D-51; Horiba, Tokyo, Japan). HSZ-385 zeolite powder (25 mg) was added and the solution stirred for 1 h at 25 °C.

**Quantitative analyses**

To determine the concentration of sulfonamide antibiotics in porcine urine, solid-phase extraction (SPE) was undertaken. The concentrations of sulfonamides were measured by a standard additional method using liquid chromatography tandem mass spectrometry (LC/MS/MS; Acquity UPLC-Xevo TQ; Waters, Milford MA, USA) after SPE.

The non-purgeable organic carbon (NPOC) content and concentrations of ions, urea and uric acid of porcine urine were measured using a Shimadzu Total Organic Carbon analyzer (TOC-5000A; Shimadzu, Kyoto, Japan), an ion chromatograph (DX-120; Dionex, Sunnyvale, CA, USA) and an Automatic Analyzer (Fuji Drichem 3500V; Fuji Photo Film, Tokyo, Japan). The details of quantitative analyses are shown in the Appendix section (see http://www.iwaponline.com/wst/067/513.pdf).

**RESULTS AND DISCUSSION**

**Adsorptive removal of sulfonamide antibiotics in pure water and synthetic urine by HSZ-385 zeolite**

The adsorption behavior of the seven sulfonamide antibiotics in distilled water on HSZ-385 at different pH conditions is shown in Figure 1(a). Adsorption efficiency was defined as the ratio of the amount of sulfonamide antibiotics adsorbed onto the zeolite to the initial amount of sulfonamides. HSZ-385 could adsorb sulfonamide antibiotics at pH 7, indicating that HSZ-385 is an effective adsorbent for removing sulfonamides from aqueous media (Figure 1(a)). We also evaluated the adsorption behavior at pH values of 8 and 9 because the pH of urine increased at ≈8–9 through the ammonification of urea. HSZ-385 showed adsorptivity for sulfonamides under alkaline conditions even though the adsorption efficiency decreased compared with that observed at pH 7. It is known that sulfonamides have cationic, neutral and anionic charges attributed to protonation and deprotonation of the amino and sulfonamide groups (Figure A2) (Boreen et al., 2004). We have also applied hydrophilic zeolites such as A-type and F-type zeolites to the adsorption of sulfonamides. However, they could not remove sulfonamides, suggesting that the adsorption of sulfonamides on HSZ-385 was based upon hydrophobic interactions between neutral sulfonamides and hydrophobic silica surfaces (Fukahori et al., 2011). The pKₐ,2 (negative log of the acid dissociation constant of the second most acidic proton) values of sulfonamides used in the present study were 5.3–7.4, implying that neutral sulfonamides are readily adsorbed in comparison with those in the anionic form.

In our previous study, a pH-dependent adsorption model was developed based on the Langmuir...
Table 1 | Composition of synthetic urine and porcine urine

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage conditions</th>
<th>Storage period (day)</th>
<th>pH</th>
<th>Cl⁻</th>
<th>NO₂⁻</th>
<th>NO₃⁻</th>
<th>PO₄³⁻</th>
<th>SO₄²⁻</th>
<th>Na⁺</th>
<th>NH₄⁺</th>
<th>K⁺</th>
<th>Mg²⁺</th>
<th>Ca²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic urine</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine urine</td>
<td>After sampling</td>
<td>0</td>
<td>7.3</td>
<td>137.5 ± 1.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>16.3 ± 0.2</td>
<td>30.8 ± 0.2</td>
<td>118.7 ± 1.8</td>
<td>18.9 ± 0.2</td>
<td>52.3 ± 0.5</td>
<td>4.9 ± 0.1</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Room temperature</td>
<td>1</td>
<td>8.9 ± 0.2</td>
<td>74.3 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>21.0 ± 0.2</td>
<td>19.7 ± 0.2</td>
<td>50.2 ± 0.5</td>
<td>15.0 ± 0.1</td>
<td>57.2 ± 0.6</td>
<td>3.1 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>without pH</td>
<td>3</td>
<td>9.0 ± 0.1</td>
<td>84.6 ± 0.7</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>17.1 ± 0.1</td>
<td>16.3 ± 0.1</td>
<td>48.4 ± 1.4</td>
<td>166.9 ± 8.5</td>
<td>57.6 ± 0.6</td>
<td>0.4 ± 0.0</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Room temperature</td>
<td>7</td>
<td>9.1 ± 0.1</td>
<td>81.0 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>17.1 ± 0.1</td>
<td>16.1 ± 0.2</td>
<td>51.1 ± 1.9</td>
<td>261.8 ± 9.6</td>
<td>58.5 ± 0.8</td>
<td>0.5 ± 0.0</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>4 °C without pH</td>
<td>30</td>
<td>9.1 ± 0.1</td>
<td>81.5 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>17.4 ± 0.2</td>
<td>16.3 ± 0.1</td>
<td>45.4 ± 1.6</td>
<td>269.8 ± 6.6</td>
<td>55.8 ± 0.7</td>
<td>0.5 ± 0.1</td>
<td>2.3 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Room temperature</td>
<td>4 °C pH 1</td>
<td>7.8 ± 0.1</td>
<td>84.0 ± 0.6</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>19.6 ± 0.2</td>
<td>16.2 ± 0.2</td>
<td>48.1 ± 1.4</td>
<td>161.0 ± 0.5</td>
<td>55.3 ± 0.5</td>
<td>1.7 ± 0.1</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>8.6 ± 0.2</td>
<td>84.6 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>18.5 ± 0.2</td>
<td>16.1 ± 0.1</td>
<td>49.4 ± 1.4</td>
<td>252.1 ± 1.1</td>
<td>58.7 ± 0.7</td>
<td>0.8 ± 0.1</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>30</td>
<td>9.1 ± 0.1</td>
<td>85.5 ± 0.7</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>17.8 ± 0.1</td>
<td>17.3 ± 0.2</td>
<td>51.1 ± 1.6</td>
<td>49.1 ± 3.7</td>
<td>58.9 ± 0.6</td>
<td>0.2 ± 0.0</td>
<td>2.4 ± 0.1</td>
</tr>
</tbody>
</table>

*SO₄²⁻ Concentration was too high due to the addition of sulfuric acid for pH control.
isotherm by consideration of the acid-base equilibria of sulfonamides; the adsorption behaviors of STZ, SMzi, SMthi, SDD and SMzl could be expressed as a function of pH (Fukahori et al. 2011). Using our model, we calculated the adsorption efficiency of sulfonamides (shown in Figure 1(a) as cross symbols). The volume of solution, sulfonamide concentration, and zeolite dosage was set as 5 mL, 10 mg/L and 25 mg, respectively. The calculated results corresponded well with the experimental adsorption efficiencies (although the experimental adsorption efficiency of SMthi was lower compared with the calculated one).

The adsorption efficiency of sulfonamide antibiotics in synthetic urine at pH values of 7, 8 and 9 was also evaluated (Figure 1(b)). HSZ-385 could adsorb sulfonamides in synthetic urine. The adsorption efficiencies were equivalent to those obtained in distilled water at all pH conditions, and ions such as sodium, potassium, chloride and phosphate did not affect the adsorption of sulfonamides on HSZ-385. Wang et al. (2010) reported that the phosphate ion inhibited the adsorption of tetracycline antibiotics in soil: the surface charge of soil particles changed through competitive adsorption of the phosphate ion. As mentioned above, we suggest that sulfonamides are adsorbed onto HSZ-385 through hydrophobic interactions; so hydrophilic ions do not seem to affect adsorption efficiency.

Composition of porcine urine during storage

It is well known that the pH of urine increases due to the formation of ammonia through the hydrolysis of urea \((\text{NH}_2\text{CO} + \text{H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{CO}_2)\) (Wilsenach et al. 2007). Conversely, from the viewpoint of the sustainable and effective use of water and resources, our research team has also been investigating the recovery of nutrients such as phosphate and nitrogen from livestock feces and urine (Fujiwara 2012). Hotta & Funamizu (2008a) studied the recovery of nutrients from human urine and concluded that the source separation of urine from feces was effective for the recovery of nitrogen in urine; urea ammonification was relatively suppressed because fecal bacteria mainly produced urease. Moreover, Hotta & Funamizu (2008b) reported the inhibition factors of urea hydrolysis in stored urine, and found that a lower pH (≈1) completely stopped urea hydrolysis.

In the present study, we collected porcine urine without contamination with feces. The composition of porcine urine during storage with or without control of pH and temperature was investigated. An increase in pH was observed in urine stored without pH control, and urea seemed to be hydrolyzed by urease in porcine urine (Table 1). In particular, in the urine stored at room temperature, pH increased immediately and reached 9 after 1-day storage. The concentration of the ammonium ion increased for 7 days and then reached a plateau, suggesting that almost all urea in the urine was...
hydrolyzed after 7 days. For urine stored at 4 °C without pH control, the pH reached 9 after 7-day storage; the concentration of the ammonium ion continued to increase until day 30, and reached an equivalent value to that obtained when stored without control of temperature and pH. These results suggested that the cooling of urine could retard (but not inhibit) the hydrolysis of urea. Conversely, pH and the concentration of ammonium ions in porcine urine stored at pH 1 hardly increased during 30-day storage. Similar to the results reported in a previous study, pH control was effective for inhibition of the hydrolysis of urea.

Except for the ammonium ions formed through the hydrolysis of urea, only the concentrations of magnesium and phosphate ions changed during storage without pH control, and precipitations were observed simultaneously. Udert et al. (2003) reported the natural precipitation of ammonium struvite (NH₄MgPO₄) in porcine urine with all available magnesium ions. The decreased amount of magnesium and phosphate was nearly equal, so ammonium struvite seemed to be formed as a precipitate.

Adsorptive removal of sulfonamide antibiotics in real porcine urine by the zeolite HSZ-385

We confirmed above that it is not ion concentrations but instead pH that greatly affects the removal of sulfonamide antibiotics. Hence, adsorption behaviors in porcine urine with different pH values were investigated. Before the adsorption test, we added HSZ-385 to porcine urine without sulfonamides and then investigated the change in urine composition. The tests were achieved using fresh urine and stored urine (RUN-1 and RUN-2, respectively, in Table 2). The ion concentrations shown in Table 2 were different compared with those shown in Table 1 because different urine types were used in each experiment. The concentrations of urea and uric acid of the porcine urine used in RUN-1 were 7.7 and 0.01 g/L, respectively. In RUN-2, the NPOC was lower compared with that used in RUN-1; this was presumably due to the mineralization of urea and other organic matter. The ion concentrations were unchanged through the addition of HSZ-385, a finding that corresponded to the result obtained using synthetic urine. NPOC slightly decreased in RUN-2. Hence, organic carbon contained in stored porcine urine may adsorb onto HSZ-385.

The adsorption efficiency of sulfonamides in porcine urine is shown in Figure 2. We expected competitive adsorption of organic matter on HSZ-385 to occur and the adsorption efficiencies to decrease, but HSZ-385 showed strong adsorption. Without the zeolite, the concentrations...
of antibiotics were unchanged. Although sulfonamide concentrations (10 mg/L each) were two orders of magnitude lower than that of NPOC (4–7 g/L), the adsorption efficiencies at pH 7 were equivalent to those obtained in distilled water and synthetic urine, indicating that HSZ-385 selectively adsorbed the sulfonamides in porcine urine. Conversely, in stored urine (pH 9), the amount of all sulfonamides adsorbed on HSZ-385 decreased compared with those obtained in distilled water and synthetic urine (Figure 1). During storage, organic matter which competitively adsorbs on HSZ-385 might form, resulting in low adsorption efficiencies in stored urine. The details of how organic matter is adsorbed on HSZ-385 are currently under consideration.

Adsorption rates of sulfonamides on HSZ-385 in porcine urine

The time-courses of sulfonamide concentrations in fresh porcine urine (pH 7) with HSZ-385 are displayed in Figure 3 (a). We have investigated the adsorption rates of sulfonamide antibiotics in pure water: high adsorption rates were observed (Fukahori et al. 2011). Similarly, in porcine urine, adsorption was rapid. In our previous study, a pseudo-second-order model was applied to evaluate the adsorption rate of sulfonamides (Azizian 2004):

\[
\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k q_e}
\]

where \( k \) (g/mol min) is the rate constant of the pseudo-second-order adsorption, and \( q_e \) (mol/g) and \( q_t \) (mol/g-zeolite) are the amount of sulfonamide adsorbed on the zeolite at equilibrium and at \( t \) (min), respectively. A pseudo-second-order model was applied to the experimental data shown in Figure 3(a) and the plot \((t/q_t)\) versus \( t \) tested (Figure 3(b)). As a measure of the adsorption rate, the half-life time \( t_{1/2} \) is frequently used; \( t_{1/2} \) is the time required to adsorb half the amount of \( q_e \). The value of \( t_{1/2} \) could be obtained by inserting \( t = t_{1/2} \) and \( q_t = q_e/2 \) into Equation (1):

\[
t_{1/2} = \frac{1}{k q_e}
\]

The values of \( t_{1/2} \) for STZ, SMzi, SMthi, SDD, SMzl, SMM and SDM were 10.4, 0.8, 18.1, 0.9, 7.6, 3.5 and 4.9 s, respectively. These findings suggested that the adsorption of sulfonamides occurred rapidly and reached equilibrium within 15 min, which is somewhat faster than that observed for activated carbon (Mestre et al. 2007).
CONCLUSIONS

The adsorption behaviors of seven sulfonamide antibiotics in distilled water, synthetic urine and real porcine urine on high-silica zeolite HSZ-385 were investigated. HSZ-385 could adsorb sulfonamides in synthetic urine. The adsorption efficiencies were equivalent to those obtained in distilled water, showing that various ions did not affect the adsorption of sulfonamides on HSZ-385. We investigated the composition of porcine urine during storage with or without control of pH and temperature. We found that the pH of urine stored at room temperature without pH control increased immediately, and reached 9 after 1-day storage. The adsorption efficiencies of sulfonamides in fresh porcine urine were equivalent to those obtained in distilled water and synthetic urine. However, in stored urine (pH 9), the amount of all sulfonamides adsorbed on HSZ-385 decreased compared with those obtained in distilled water and synthetic urine. These results suggest that the antibiotics contained in livestock urine should be treated immediately after excretion.

REFERENCES

Ternes, T. A. 1998 Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 32 (11), 3245–3260.

First received 26 January 2012; accepted in revised form 19 July 2012